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HANDBOOK

FOR THE

PHYSIOLOGICAL LABORATORY

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PHYSIOLOGICAL LABORATORY

BY

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TO

WILLIAM SHARPEY, M.D. LL.D. F.R.S. F.R.S.E.

PROFESSOR OF ANATOMY AND PHYSIOLOGY IN UNIVERSITY COLLEGE, LONDON, &c. &c.

Dear Dr. Sharpey,

To you, who have been these many years the friend of physiologists throughout the world, and who, by your original work, by your teaching, by your generous aid and judicious counsel, have been the mainstay of physiology in England, we desire to dedicate this attempt to promote the study of our science.

Accept it as a token of our personal regard, as well as of the high value we set on your life-long labours.

Your devoted Friends,

MICHAEL FOSTER,
J. BURDON-SANDERSON,
T. LAUDER BRUNTON,
E. KLEIN.

EDITOR'S PREFACE.

This book is intended for beginners in physiological work. It is a book of methods, not a compendium of the science of physiology, and consequently claims a place rather in the laboratory than in the study. But although designed for workers, the authors believe that it will be found not the less useful to those who desire to inform themselves by reading as to the extent to which the science is based on experiment, and as to the nature of the experiments which chiefly deserve to be regarded as fundamental.

The practical purpose of the book has been strictly kept in view, both in the arrangement and in the selection of the subjects. Many subjects are entirely omitted which form important chapters in every text-book. They have been left out either because they do not admit of experimental demonstration, or because the experiments required are of too difficult or complicated a character to be either shown to a class or performed by a beginner.

The mode of arrangement will be found to be somewhat different in the four sections into which the work is divided. This difference, although in part attributable to difference of authorship, is mainly due to the peculiarities of the modes of demonstration required in the several subjects.

As regards the physiology of nerve and muscle, it is sufficient to refer the reader to the author's introduction for an exposition of the method followed. In the histological part will be found a purely objective description of anatomical facts and methods. Substituting chemical for anatomical, the same thing might be said of the chapters relating to the chemical functions. Here, where minuteness of description is essential, great pains have been taken to give the student the most ample details as regards materials for work, instruments and methods. In the chapter on the blood, the same object has been kept in view, but in those relating to the mechanical functions of circulation and respiration, where either man or the higher animals must be for the most part the subjects of observation, and where consequently the conditions of experiment are complicated by the interference of the nervous system to an extent which it is often difficult to estimate, it has been found impossible to avoid entering somewhat more largely into theoretical explanations.

In the chapters on digestion and secretion, and in the remainder of the chemical part, those experiments or methods which are most important and best suited for demonstration are distinguished by two asterisks (**), the less important by a single asterisk (*). The absence of an asterisk at the beginning of a paragraph denotes either that the experiment is unimportant or that it is difficult to perform. A dagger (†) is used to draw special attention to a test or procedure.

I have to record Dr. Brunton's obligation to Dr. Arthur Gamgee, F.R.S., for many important suggestions in the preparation of the chapter on secretion. Dr. Brunton further wishes me to state that, although he has recommended no method as suitable for demonstration which he has not himself tried, he has freely availed himself of the well-known works of Hoppe-Seyler, Gorup-Besanez, and Kühne, both in the arrangement of the sections and in the selection of experiments.

It has been judged expedient by the Publishers to separate the illustrations from the text. In this way full justice has been done to the engravings of the Histological part, which have been executed by Mr. Collings from the original drawings of the author.

Most of the illustrations of the Physiological part are the work of the same artist, both as regards drawing and engraving. Of the remainder, several have been borrowed (with the kind permission of the author) from Mr. Sutton's work on Volumetrical Analysis.

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HISTOLOGY.

By Dr. E. KLEIN.

PART I.—PREPARATION OF THE ELEMENTARY TISSUES.

CHAPTER I.

BLOOD CORPUSCLES.

In the microscopical examination of the blood, we have to do only with the study of the formed elements, namely the colourless corpuscles and blood disks.

Colourless Blood Corpuscles.—The colourless corpuscles are elementary organisms which are endowed with the power of spontaneous motion. This power belongs to them in virtue of the material of which their bodies are composed. This material is protoplasm. Their motion is of two kinds; it consists of change of form and change of place. The latter results from the former. As movements of this kind are seen in greatest perfection in rhizopods and amœbæ, they are called amœboid.

Amœboid Movements of Colourless Corpuscles.—Very active movements are seen in the colourless blood corpuscles of the newt. The cells are large and easy of observation. It is of the first importance, in beginning our study of them, that they should be placed under conditions which, if not identical with, are not materially different from, those under which they actually exist. The simplest method is the following:—

Take a clean glass slide and an absolutely clean cover-glass, which, as we must use high powers (that is, objectives of which the focal distance is short), must be thin. Take the newt out of the water, dry the tail, cut off its end. If no blood comes, squeeze the organ from the root towards the tip until a drop is obtained. One of two methods may now be used: 1st, let the blood drop upon the middle of the glass slide, and place the cover-glass on it in such a way that one edge rests on its surface, while the opposite edge is supported by the finger or forceps. Then let the glass gradually down upon the drop. Or, 2ndly, collect the blood on the cover-glass by bringing it into contact with the drop, then place it on the slide with its clean surface upwards. By following either of these methods, the introduction of air-bubbles will be avoided, which would otherwise be a source of difficulty to the beginner. The drop should be neither too small nor too large. The following inconveniences arise from its being too large:—1. The thin cover-glass does not lie steadily in its place, but floats on the drop in such a way that, by the slightest movement of the table, currents are produced in the liquid which render observation difficult or impossible. 2. If it is desired to keep the preparation for a length of time under observation, it is necessary to adopt some means to prevent the liquid from evaporating; for, if this is not done, it gradually dries from the edges, and soon becomes unfit for the observation of amœboid movements, we therefore enclose the preparation in oil, as will be immediately described, and experience shows that, by so doing, the movements may be watched for twelve hours or more continuously—a time which is sufficient for a complete study of the phenomena in question. This, however, cannot be done unless the drop is very small. 3. If high powers are used, the front glass of the objective comes into contact with the cover-glass, and produces currents due to pressure.

If, on the other hand, the drop is too small, the elements are pressed upon by the cover-glass, and thereby subjected to unnatural conditions. No definite rule can be given as regards the size of the drop, which must of course vary with that of the cover-glass.

The mode of surrounding a preparation of blood with oil is as follows:—Take a drop with a glass rod or camel-hair pencil, and let it fall at the very edge of the cover-glass in such a way that, although most of it is on the surface of the slide, it covers a little of the cover-glass also. Then incline the glass slide slightly, and with the rod lead the oil from the drop along the edge of the cover-glass, taking care not to press upon the latter. If one drop of oil is insufficient, of course another must be added. Take great care to avoid smearing the cover-glass too far; for by so doing, the space available for observation may become inconveniently limited.

Having thus obtained a preparation of blood entirely protected from evaporation, we are ready to begin our study of the colourless corpuscles.

Varieties of Colourless Corpuscles.—As soon as we have brought a field containing blood into focus, we see, in addition to a multitude of coloured blood disks, to which we at present pay no attention, a greater or less number of colourless corpuscles, which themselves differ from one another both in size and aspect, and in their property of spontaneous movement. Three forms may be distinguished, which we will examine in succession:—

1. **Common Large Colourless Corpuscles.**—Supposing that a few moments have elapsed since the preparation was made, some of these pale corpuscles are sure to be seen floating hither and thither in the liquid with a rolling movement. They are much more numerous than the other forms.

Fix the attention on one of these bodies, and observe, first, that it is so transparent that, as it rolls over and over, a single granule embedded in its substance may be kept constantly in view. Continuing the observation, notice that the surface of the corpuscle, at first smooth, gradually becomes uneven. The cause of the unevenness is this. The surface is beset with a greater or less number of filamentous appendages, varying in length, and distributed over the surface with variable uniformity. These seem to consist of the same material as the body of the corpuscle. When they are short they may be compared to prickles, when longer they are often bent at the point. Sometimes we see one of the processes lengthen itself, while another disappears; sometimes a whole group of processes push out on one side, while others are retracted on the opposite side. Occasionally, from the smallness and great number of the processes, it is scarcely possible to be sure as to the changes which occur. Here is a corpuscle which appears to be gradually enlarging. Let us follow the changes it will undergo. Already it covers a space three or four times as great as before. Simultaneously with this increase of size, its form becomes irregular and (as may be ascertained by the fine-adjustment screw) its vertical measurement is diminished, so that it now constitutes a thin layer limited by a distinct contour. Soon, however, the circumference thins out in certain directions, so that the edge can no longer be discerned; the only evidence of its existence in these attenuated parts being that the field appears to be covered with a granulous film.

In the layer of protoplasm we have now before us, some parts are hyaline, or contain at most a few minute granules. In others, you will notice, there are clear spots with well-defined contours, which differ indefinitely in size, and have no definite arrangement. Many of them are so clear that they look like perforations. It is characteristic of them that they are undergoing change, both as regards their relative position and relative distinctness, some coming into view while others are fading. These we call *vacuoles*. They are believed to be cavities filled with liquid, the origin of which is due to the constant commotion of the protoplasmic mass. If this be so, it is easy to understand why it is that they appear and disappear so rapidly. We next observe that at some part of the corpuscle (often, but not always, towards the centre) one or more bodies may be distinguished of roundish, ovoid, or irregular form, and tolerably distinct contour, somewhat less refractive than the surrounding protoplasm, and containing one or more granules. These bodies are commonly close together, and are called *nuclei*. The nuclei are usually invisible so long as the colourless blood corpuscle is spheroidal; when it spreads out into a layer, they can be distinguished. But they can also be observed when the lamina draws itself together into an irregular clump; and it may be then seen that they are subject to continual change, both as regards form and relative position.

We now leave the corpuscle we have been hitherto studying and observe another, which is roundish, and exhibits a very few delicate processes. At present we see no nuclei. After a time we notice that one of the processes suddenly becomes longer and thicker, so that the corpuscle is now club-shaped, consisting of a tapering stalk ending in a knob. The stalk encloses an oblong, compressed nucleus, and the knob two such nuclei close together, the surfaces of both being shaggy, with minute processes. We have not long to wait until the body loses this form. A new process, towards which the two nuclei tend, shoots out from the knob, at right angles to the stalk. The knob becomes smaller in proportion to the growth of the process, while the two nuclei gradually approach its extremity. The next change is, that each process lengthens out in the direction of its axis into a filament, the two together being of such a length as to stretch over the whole field. These filaments spring from a small clump of granular protoplasm—the original knob above mentioned. Each filament swells out at its end into a little mass, which, in the one case, contains a single nucleus, in the others, two nuclei. Continuing our observation, we notice that the clump at the junction of the two filaments disappears, while the other masses, which are now united by a straight thread of nearly equal thickness throughout, get larger, and send out new processes. The larger mass now creeps nearer the edge of the field; the smaller is drawn after it, but moves more slowly, so that the hyaline thread which connects them gets thinner and longer. But while we are watching it, the large mass undergoes changes which are a repetition of what we before observed in the original clump. A process shoots out from it at right angles to the direction of the thread: into this process one of the nuclei finds its way; it then stretches out into a filament, which is swollen at its extremity into a protoplasmic envelope for the nucleus. Still later, we find that the filaments become thicker and shorter; that the clumps between which they stretch, again approach one another, until, in their confluence, the original form reappears. A similar series of changes may be witnessed in any corpuscle of the kind we have been studying.

2. **Granular Corpuscles.**—Of the three kinds of pale corpuscles which, as before stated, are to be observed in the blood of the newt, we have now to consider the granular cells. These are larger, but much less numerous than the others, and are distinguished by the large dark granules they contain. To observe them we must make a fresh preparation, for they undergo changes of form much more rapidly than the others. The granular corpuscle is at first spheroidal. Very soon its surface exhibits round and entirely hyaline prominences, into which, however, granules appear shortly to find their way. So long as the corpuscle presents this appearance, the only changes of form observable consist in heaving movements of the prominences. Eventually, one of these suddenly shoots out into a prong-like process, into which the granular mass of the original cell flows. Soon the corpuscle throws out a second similar process, into which the mass again gathers itself, and in this way advances across the field, with more or less rapidity. After this has gone on for a certain time the movements change their type: the corpuscle lengthens itself out into a thread, in which the movement of the protoplasm is rendered visible by that of the dark granules which it contains. The thread swells out at the end into a little mass, from and towards which alternately the rolling motion of the granules is seen to be directed. Often a granular corpuscle may be observed to creep about among groups of coloured blood-disks, stretching out its process with the terminal knob, as if this were a feeler. In other cases we may witness the whole series of changes described in the preceding paragraph as occurring in the ordinary form of colourless blood corpuscle; the main difference being that the transformations are accomplished within shorter periods. Finally, it may be noticed that in granular cells, even when they are spheroidal, the nuclei often show themselves as ovoid spaces free from granules. They are, however, much more readily distinguished after the cell has undergone changes of form.

3. **Colourless Corpuscles of the third form.**—In addition

to the common colourless corpuscles and the granular cells we have just had under observation, we notice a considerable number of colourless elements of a different character. These are of three kinds: (*a*) Small, well-defined bodies, resembling nuclei, which retain only for a very short time the spheroidal form which they had at first; (*b*) larger corpuscles, consisting of finely granular protoplasm, with jagged outline, containing three or four distinct nuclei, which may be either roundish, or flattened against each other, exhibit a double contour, and contain a few fine nucleoli which are relatively of large size, so much so, that they often appear to be surrounded by a narrow zone of protoplasm; (*c*) large masses of finely granular protoplasm, which commonly are of irregular form, and enclose bodies similar to the nuclei above described, varying in number from five to twenty in each mass.*

Methods of Warming a Preparation.—As in our further study of the blood corpuscles it will be necessary to employ artificially increased temperatures, we proceed to describe the methods employed for applying heat to preparations whilst under microscopic observation. These methods are of two kinds. The first is used when we wish to subject the preparation for an indefinite period to an increased temperature, to which it has been gradually raised; the second when we wish to warm it suddenly, but for a very short period. To accomplish the first of these objects, a very simple contrivance, shown in Fig. 1, may be used. Take a cover-glass, and spread all round the edge of its upper surface a thin layer of oil; then take another cover-glass of the same size as the first, place on its centre the drop of the blood to be examined, and allow it to fall on the glass previously prepared, edge to edge, with the blood drop downwards. The drop will then occupy the space between the two, enclosed by the layer of oil in such a manner that it may be examined under high powers. The preparation may then be readily lifted with the aid of a lancet-shaped knife, and placed on the orifice of the copper plate (*e*). The copper rod (*g*) is then gently warmed by means of a spirit-lamp, a little cacao butter (or some other fat, the fusing point of which nearly corresponds to the desired temperature) having been previously placed on the copper plate, close to the preparation. As soon as the cacao butter begins to liquefy, the flame of the lamp is diminished, or the lamp itself is removed to a greater distance, until the heat communicated by it to the plate through the rod is just sufficient to keep the fat from solidifying. If it is desired to employ higher temperatures, or to measure the temperature with greater exactitude, it is necessary to have recourse to Stricker's warm stage.

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* Free nuclei of coloured corpuscles, which may be seen if the preparation has been subjected to pressure, must not be confused with these structures.

Stricker's Warm Stage.—Of this there are two forms. In one the mode of heating, and consequently of modifying the amount of heat communicated, is that which has been already described (see Fig. 2). From its simplicity it is well adapted for the beginner, while it enables the more practised observer to maintain any desired temperature within very inconsiderable limits of variation. The other, in addition to the greater exactitude which can be attained, has the advantage that, by its aid, it is possible to continue the observation for a very long period. It is this which is employed by Sanderson and Stricker for the study of the circulation in mammalia. For our present purpose we do not require the whole apparatus, so that it is only necessary to refer to those parts of it which are shown in Fig. 3.

In the employment of this apparatus several difficulties are encountered. For instance, the temperature of the water receptacle is only in part controlled by the regulator. Then, again, the temperature of the stage is subject to variation according to the rate at which the water flows into and escapes from it; so that, if great care be not taken in the adjustment, constancy cannot be relied on. Another practical difficulty lies in the fact that the temperature of the water in the receptacle is different from that in the stage, the rate of flow being so inconsiderable that there is necessarily a great loss of heat by radiation from the metal surface. If the stage be not fitted with a thermometer, this difference of temperature may be determined, once for all, by comparative measurements, so that the true temperature of the stage can then be known at any time by deducting the ascertained loss of heat, *i.e.*, the ascertained difference above referred to, from the temperature to which the regulator is adjusted.

Method of varying the temperature rapidly.—In connection with this apparatus, it is convenient to describe the method employed for subjecting a preparation to sudden alterations of temperature. With this view the following contrivance is used: —A clip is placed on the tube leading from the water receptacle (*C*, Fig. 3), by means of which the access of warm water to the stage may be interrupted. The end of the escape-tube (*D*) is then allowed to dip into a vessel of cold water. This done, cold water may be readily introduced into the stage, so as to cool it suddenly, by suction through the tube (*C*), which must be provided with a branch (not shown in the figure) between the clip and the stage, for the purpose. This, of course, at once lowers the temperature. To effect a sudden rise, all that is necessary is to open the clip. For short experiments, it is not necessary to have a water receptacle specially constructed for the purpose; a large flask, supported over a lamp, and without a regulator, may be substituted for it, provided that, in addition to the discharge-tube, a thermometer is passed through the cork, in order that the variations of temperature may be observed, and the application of heat modified accordingly.

Effects of Warmth on the Colourless Corpuscles.—We now return to the study of the drop of newt's blood, enclosed between two cover-glasses, with which we were occupied. On subjecting the preparation to a temperature of 38° C., the first fact that we notice is that the movements of the colourless corpuscles in general, and of the granular ones in particular, are much more active. We shall not, however, occupy ourselves at present with these, but shall direct our attention to the three kinds of corpuscles which we have included in our third division.

On the warm stage we may observe in these bodies (which differ only in size) two kinds of change. One of these consists of alteration in the form of the protoplasm, from the surface of which processes shoot out in all directions. This is more particularly seen in the forms we have designated *b* and *c*. In the form *a*, although the nucleus at first appears bare, it is afterwards seen to be surrounded by a protoplasmic envelope; this may throw out a pointed process, which, after stretching out to a considerable length, is retracted, to be succeeded by others. If the preparation is kept for a length of time at 38°, the elements of the form *a* undergo other remarkable alterations. They become strongly refractive, lose their double contour and sharply-defined aspect, and acquire a form which, at first globular, subsequently exhibits constrictions; so that they become in succession kidney-shaped, dumb-bell shaped, and rosette-shaped, until they eventually assume a nodulated aspect. In the course of the process it is common to observe the furrows or constrictions forming, disappearing, and reappearing repeatedly; but, sooner or later, they become more and more distinct and complete, so that the body assumes the appearance of a clump of highly refractive minute globules. Considering the coincidence of the changes of form and aspect of the nucleus with those which occur simultaneously in the cell, it is scarcely possible to doubt the dependence of the former upon the latter, especially if we bear in mind the concomitant changes in optical properties. So that we must regard these appearances as indicating that the nuclei take an active part in the changes of form.

In the form *c* the cell-substance itself may be also the seat of a process of division. In one instance at least I have, of course after many hours of observation, witnessed the division of a cell which originally contained five nuclei. The cell in question in the first place exhibited a transverse furrow: this became deeper and deeper, so that, eventually, two masses were formed, united together by a neck, the smaller containing two nuclei, the larger three. These nuclei had already undergone the process of cleavage above described. By the lengthening, thinning out, and final rupture of the isthmus, the two corpuscles came apart. In the larger of the two, which was now exclusively observed, there appeared gradually two boss-like prominences, each of which contained a number of small bodies resulting from the cleavage of the nuclei. By the constriction of the base of each of these prominences it gradually separated from the rest of the cell. One of them, after separation, sent out a process; in the other, no alteration of form could be observed. It is probable that the forms *a* and *b* are the offspring of *c*.

On the warm stage, division can also be observed in the first and second variety of colourless corpuscles. Thus, for example, it sometimes happens that the process described only results in actual separation by rupture of the filament. In other cases a corpuscle undergoes division by a process of cleavage, preceded by the repeated formation, disappearance, and reappearance of furrows. In all cases of real division it is to be observed that the young cells produced exhibit very active movements, changing thereby in form and place.

Colourless Corpuscles of Man.—The mode of examining the colourless corpuscles of other classes of animals is similar to that above described. It is, however, necessary to add some observations as to the characters which these bodies present in human blood. A drop of blood, taken from the finger, is placed between two cover-glasses, as above described, and examined on the warm stage at a temperature of 38° C. The human colourless corpuscles are smaller than those of the newt, and exhibit much less variety in their appearance. They are either quite pale, or they contain a variable number of dark granules. The movements are less active than those of newt's blood, but sometimes are comparable with them. When they are more active than usual, the mode in which their processes are thrown out and retracted, and the characters of their progressive movement correspond with the descriptions already given. On one occasion I have observed movements which were even more lively than those commonly seen in the newt, and resembled those of rhizopods in the extreme rapidity with which the successive protrusion of processes, and corresponding interstitial fluxion of the protoplasm occurred. This happened in the case of a patient suffering from hæmorrhagic anæmia.

Feeding of Colourless Corpuscles.—We have now to study the faculty possessed by the colourless corpuscles of taking, by virtue of their amœboid movement, solid particles into their substance. For this purpose we employ either finely-divided fatty substances or colouring matters. The subject is of great interest in relation to the mode in which amœboid cells take in nourishment. To the histologist it is further of importance, as affording him a means by which to mark individual corpuscles, so as to follow them in their wanderings through the organism. The materials used are the following:—*a.* **Vermilion.** This is prepared by prolonged trituration in a half per cent. solution of common salt. *b.* **Carmine.**—Carmine is dissolved in as little *liquor ammoniæ* as possible, in a small beaker, and filtered. Common concentrated (commercial) acetic acid is then added with agitation, until a drop of the mixture, when examined under a low power, is seen to contain granules. If too much is added, the precipitate is not fine enough. The latter is then to be separated by careful decantation, and suspended in a half per cent. salt solution as before. It is well to dilute the liquid, with its bulk of serum before using it. *c.* **Aniline Blue** is dissolved in common methylated spirit, and filtered. Water or salt solution must then be added gradually, so as to obtain a fine precipitate, the resulting liquid being mixed with serum as above. *d.* **Fresh Milk.**

If it is intended to watch the process of feeding, a small drop of blood, to which one of the liquids above mentioned has been added, is examined, either in the ordinary way, in the case of amphibian blood, or on the warm stage if mammalian blood is employed. If our object is merely to observe corpuscles already fed, the liquids in question may be injected either into the jugular vein (of rabbits or guineapigs) or into the abdominal vein (of frogs), care being taken to employ a sufficiently large quantity. After 10–30 minutes, a drop of blood may be taken for examination. (See Chapter VII., as to injection into the veins, and Chapter VIII., as to the lymphatic system.) Whichever plan is adopted, it is alike possible to satisfy ourselves that the cells not only take in foreign bodies, but that they also have the faculty of discharging them, and further, that when one cell comes into contact with another, it often gives up to it the solid bodies which it has itself before ingested. In general, the tendency to ingestion varies with the activity of the amœboid movement, for the first thing observed is an adhesion, either of the surface of the central part of the corpuscle, or of a process to the foreign body, followed by a retraction of the adherent part into its substance.

Application of Liquid Reagents.—It is, in the first place, of importance to ascertain what liquids can be added without affecting the vital phenomena of the colourless corpuscles. Such are designated by the adjective *indifferent*, and are those which are always to be used in the study of fresh living tissues. For example, we may use fresh serum or transudation liquids, as also the aqueous humour of the eye, which has the important advantage of being entirely free from formed elements. The most commonly used indifferent liquid is the half per cent. solution of common salt already mentioned, which is of great value; although, as may be readily understood, it is not altogether without action on living tissues. In the examination of blood, it is added as a preparatory step to the addition of other reagents. With this view the solution is dropped from a capillary pipette (Fig. 4) upon a slide; a drop of newt's blood being then added to it and covered. It is seen that the colourless corpuscles have undergone no material change, but that, in some instances, their movements are not quite so active. The coloured corpuscles, which in our previous examination we have disregarded, are now seen as smooth oval elliptical disks, which, when looked at edgewise, present an outline as if they were oblong rods. Those which lie horizontally look, for the most part, like greenish-yellow bodies of oval form; in some of which we can distinguish a central elliptical nucleus. Soon, changes occur, in consequence of which the colour becomes unequally distributed, the margins are more or less curved, or the surfaces marked with what look like folds. These appearances are referable probably to a process analogous to coagulation.

Method of Retarding Evaporation.—If it is intended to keep a preparation of this kind long under observation, it is necessary to add saline solution from time to time from a pipette. If, however, as is often the case, it is of importance to keep an individual corpuscle in the field, this method cannot be employed without great risk of the object being carried away by the stream. To avoid this result, it is a good plan to place a drop or two of solution near each of two opposite margins of the cover-glass. By these drops the liquid under the glass is preserved from evaporation, because the space in the immediate neighbourhood of the margin is kept saturated with moisture.

We may now proceed to study the action of other reagents on blood already treated with saline solution. We use the so-called **method of irrigation.** On one side of the cover-glass a small strip of blotting-paper is placed, while the reagent is discharged from the pipette at the opposite edge. When the paper has become saturated with liquid it is replaced by another, and the process repeated, so that a constant current is maintained through the preparation. If the coloured corpuscles are the special subject of study, it is best to wait until they have shrunk, for we are then sure that many of them will have had time to sink and adhere to the surface of the slide. If this precaution is neglected, they are apt to be swept away by the current.

Action of Distilled Water.—In blood preparations irrigated with distilled water, the movements of the colourless blood corpuscles gradually cease. The inequalities, corresponding to the processes, disappear, while the corpuscle enlarges, and assumes the globular form. From one to four (or even more) round vesicular nuclei come into view. Soon the nuclei coalesce to form a single mass, also having a vesicular character, which not unfrequently exhibits a rotatory movement within the corpuscle. The substance which surrounds the nucleus is pale. It contains numerous distinct granules, which show active Brownian movement. It not unfrequently happens, that a much-swollen spheroidal corpuscle, after remaining a length of time in its place without change, is torn away from its attachment to the glass by the current, in which case it may either divide into two masses, one of which continues adherent, while the other floats away, or it may float away *en masse*, leaving behind it a long filament, by which it is still connected with its original point of adhesion. By renewing the irrigation, the filament will probably be severed. It is thus proved that the colourless corpuscle consists of a soft viscous substance.—The final result of the action of water on the colourless corpuscles is always disintegration; the mass suddenly disperses into the surrounding medium, all that remains of the previously so active entity is a collapsed, formless clump, in which one or two motionless granules may be seen.

In the coloured blood disks, the first change is that their surfaces become smooth, their contour becomes circular, the nucleus rounder and brighter than before, the corpuscle paler and paler, until its outline is scarcely distinguishable. Two phenomena are worth noticing before we proceed further. The first is, that, at the commencement of irrigation with distilled water, it occasionally happens that, immediately their surfaces have become smooth, the corpuscles suddenly assume a rounder and smaller appearance, and are more intensely coloured; quickly returning, however, to the elliptical form, and losing their colour as before. The second will be explained later: a coloured corpuscle appears to have separated into two parts, a pale elliptical disk and a yellow mass, occupying a central, or, more frequently, an eccentric position within it, from which coloured processes often stretch out like rays toward the periphery.

Stricker's Method.—There is another method of studying the action of water on the coloured corpuscles. For this purpose we require the warm stage (Fig. 2). A drop of water is placed on the floor of the chamber, and on the middle of the surface of the cover-glass a drop of blood, either pure or diluted with salt solution. The cover-glass is then inverted over the chamber, the edges of which have been previously oiled, or surrounded with a ring of putty, so that it is air-tight. By warming the copper wire the water is made to evaporate from the floor of the chamber, and becomes condensed on the under surface of the cover-glass. In this way we are enabled to study the gradual action of water on the corpuscles very advantageously.

Action of Salt Solution on the Blood Corpuscles of Mammalia.—In mammalian blood which has been diluted with salt solution, the naturally bi-concave coloured corpuscles exhibit a remarkable alteration, which consists in their assuming a form very similar to that of the fruit of the horse-chestnut. In those corpuscles which present their surfaces, the processes which project from the margin look like the rays of a star, while those which spring from the surface appear as dark points. In such a preparation it is not difficult to float away the coloured disks altogether, by irrigating it immediately with salt solution. The colourless corpuscles sink very rapidly, and stick to the glass, while the coloured disks remain suspended.

Let us seek for a field in which one or two colourless corpuscles only are to be seen. By discontinuing the irrigation, at the same time replacing the bit of blotting-paper so as to withdraw the fluid, we bring the cover so near the slide that it compresses the corpuscles, which in consequence appear paler and larger. The paper is now taken away, and salt solution added at the opposite edge as before. The corpuscles at once become smaller and more globular, and seem to contract; but, immediately after, dilate again, as if they were relaxing. In the resumption by the corpuscle of its original form after compression, we have to do with a phenomenon which can only be explained on the supposition that the colourless corpuscle is elastic. The nature of the contraction and the subsequent relaxation lead us, however, to suppose that the contraction is, at least partly, a result of the excitation produced by the irrigation with saline solution.

Action of Water on Mammalian Blood.—As regards the action of water on the corpuscles of mammalian blood, there is not much to be added to what has been said with reference to newt's blood; the colourless corpuscles discontinue their movements, become globular in form, exhibit vesicular nuclei and vibrating granules, and finally are disintegrated. The coloured disks lose their horse-chestnut form, become smooth and pale, and eventually disappear.

Action of Acids.—The general action of acids is so uniform that it is not necessary to refer separately to each. We content ourselves with describing the action of acetic acid. A special action of boracic acid will be noticed further on. The final result of the action of acetic acid on the blood corpuscles is the same, whether it is diluted or concentrated. The rapidity with which the changes take place is, however, different. It is always better to begin with dilute acid. If a salt solution preparation of newt's blood is, after the shrinking of the coloured corpuscles, irrigated with a liquid containing one per cent. of the ordinary commercial acid, we observe, first, that the movements of the colourless corpuscles cease, and that they enlarge and display their nuclei as sharply-defined bodies, beset with granules. If the action of the acid has been prolonged, each corpuscle appears to consist of two parts—a distinctly granular mass, which immediately surrounds the nucleus, and a bright transparent circle, with sharp outline, within which that body is enclosed. The nuclei are furrowed in such a way that their form is very variable, and, if the action has lasted long enough, they look as if actually split into smaller particles. The coloured corpuscles again become smooth, swell out somewhat, become cellular in their contour, just as after the addition of water, each showing an oblong granular nucleus, which is at first smooth, subsequently uneven and rough. Many of the blood disks return to their original elliptical form. All eventually lose their colour, but possess, even when entirely colourless, a much more distinct contour than those which have been acted upon by water. Occasionally, it happens that the nucleus becomes stained with colouring matter, and assumes a yellow tint. In human blood, the colourless corpuscles exhibit, after the action of acetic acid, the appearance of globular bodies, in which two, three, or more small shrunken nuclei are visible. The coloured disks lose their stellate form and their colouring matter, but their outlines are still distinct.

Action of Alkalies.—If a salt solution preparation is irrigated with an alkaline liquid, whatever be the source of the blood used, the colourless corpuscles at first swell, and then rapidly disappear. The coloured disks also swell out at first—those of mammalia becoming often what German authors have designated *napfförmig* (cup-shaped); eventually they lose their colour and disappear.

Action of Boracic Acid.—We have now to describe a reaction which, especially in the blood of the newt, is of importance, as serving to illustrate the intimate structure of the coloured blood disk. The action of a two per cent. solution of boracic acid on the colourless corpuscles in general, and on the blood disks of mammalia, does not differ from that of other weak acids. If, however, a salt preparation of newt's blood, in which the coloured corpuscles have already shrunk, is irrigated with the solution in question, we observe that those bodies swell and acquire a circular contour, showing, at the same time, a pale *oval* nucleus. It is now seen that, as the disk gradually pales, the nucleus becomes more and more spheroidal and yellow, while, at the same time, it increases in size. At first it is smooth, subsequently uneven. Here and there corpuscles are met with in which the yellow central body (*zooid* of Brücke) is not round, but beset with processes which stretch like rays towards the periphery. Occasionally, it can be made out that the processes are withdrawn, so that the yellow centre acquires a roundish form. The zooids eventually lose their central position, and if the preparation is protected from evaporation for a sufficient length of time, the observer is sure to see many corpuscles in which they lie, some partly, some entirely outside the outline of the pale disk. The latter (again following Brücke) we designate *œcoid*. Brücke teaches that the zooid consists of the nucleus and the hæmoglobin; that it withdraws from the œcoid which it previously, as it were, inhabited, and collects itself around the nucleus, so as to form an independent individual, capable of a separate existence. In describing further on similar appearances observed during the action of carbonic acid gas, we shall suggest another explanation of the phenomenon.

Action of Tannin on Human Blood,—Roberts' Reaction.—The action of tannin on the coloured corpuscles of human blood resembles that of boracic acid on newt's blood. When two per cent. solution of tannin is added to human blood, the corpuscles which have been already rendered star-shaped by salt solution, acquire an even contour. Soon after, a sharply-defined, yellowish-green roundish body is seen, either just within or at the margin of each corpuscle, or even outside of it, while the corpuscle itself has become colourless.

Action of Gases on the Blood.—For the study of the action of oxygen and carbonic acid gas on the blood corpuscles, either of the movable stages represented in Figs. 2 and 3 may be used. Around the edge of the central chamber we form an annular wall of putty. We then make on a cover-glass a preparation of newt's blood, to which about half its volume of distilled water has been added. The glass is then inverted over the chamber (upon the floor of which a drop of water has previously been placed) with the preparation downwards, so that its entire periphery presses evenly upon the putty ring.

The chamber is thus converted into an air-tight cavity. In Fig. 3, two tubes (H, I), with india-rubber connectors fitted to them, are shown, both of which communicate with the chamber in such a way that when it is closed above and below, a stream of gas passing in by the one, escapes by the other. By means of an apparatus in communication with the tube H, the construction of which will be readily understood from Fig. 5, the observer is able to fill the chamber at will with carbonic acid gas or with air. This is accomplished as follows :—

If the bottle containing hydrochloric acid is raised, the clip *n* opened, and the india-rubber tube *a* shut between the teeth, the carbonic acid, which is developed in M, after it has passed through the wash-bottle V, flows into the chamber, and is discharged by the tube *b*. By proceeding in this manner one hand is left free, and can be used for adjustment. To interrupt the current of gas, all that is necessary is to close N and to let down the bottle. The carbonic acid gas in the chamber is easily replaced by air, by aspiration through the tube *a*.

Action of Carbonic Acid Gas.—The preparation having been brought into focus, the gas is allowed to pass through the chamber for a short time. At first, the only observable effect is that the nuclei of the slightly smoother disks are more distinct. If the carbonic acid is now replaced by air, the nuclei again become indistinguishable. We have to do, therefore, with a transitory coagulation of the substance surrounding the nucleus. An excess of the gas brings the nuclei permanently into view. If, however, we first add to our preparation a quantity of water, sufficient not merely to swell the coloured disks, but to deprive them partly of their colour, the result is somewhat different. After a short action of the gas, the appearances are much as they have been already described; but, if an excess is admitted, bodies similar to the zooids above described as produced by the action of boracic acid, come into view.

Instead of the pale oblong nuclei, the areas of the decolorised disks enclose relatively large, yellow, roundish bodies, both the areas and the enclosed bodies being beset with fine granules. In those disks which have previously lost their colour, and are consequently scarcely visible, the nuclei become visible after the addition of excess of carbonic acid, as pale granulous bodies, the disks themselves also containing numerous granules. If we now replace the carbonic acid by air, the corpuscles recover, in every respect, their previous aspect; those in which the zooids had come into view becoming smooth, and of uniform colour, so that neither nucleus nor granules can be distinguished. Those disks which have lost their colour by the action of water become, as before, uniformly pale and indistinct. The experiment may be repeated several times. It is not difficult to explain all these appearances by coagulation.

It is a very good plan, in order to study the action of carbonic acid on newt's blood, in all degrees of dilution, to examine a salt solution preparation of such blood on the movable stage (Fig. 2), which also serves the purpose of a gas chamber. On warming the metal rod, watery vapour is disengaged from the floor of the chamber (into which a drop of water has been previously introduced), and acts upon the corpuscles.

In order to study the action of carbonic acid on the coloured corpuscles of man, it is best to employ a drop of blood mixed with salt-solution, taking care that the individual cells are as much as possible separate from one another. If, as soon as the corpuscles become horse-chestnut shaped in consequence of the action of the salt-solution, the preparation is subjected to the action of the gas, we at once observe that the acuminate projections on the surface of the corpuscles become less marked in consequence of the levelling up of the intermediate parts; and, although there are many which do not resume the biconcave form, being still saucer-shaped, they all have even surfaces. If the carbonic acid is replaced by air, the corpuscles again become horse-chestnut shaped. This reaction may also be witnessed several times in succession. The disappearance of the stellate form may be explained on the supposition that a spontaneously coagulated constituent is redissolved under the action of carbonic acid. Colourless corpuscles show their nuclei when acted on by carbonic acid, but are otherwise unaltered.

Action of Electricity.—If it is intended to subject blood to the action of electrical discharges, or of the constant or interrupted current, we place a small drop of blood on the slide (Fig. 6) in such a position that, when it is covered, it spreads between the two poles of tinfoil, which we connect by means of either of the appliances shown in the figure with the secondary coil of the induction apparatus.

According to Rollett, it is advisable, in using *electrical discharges*, that the tinfoil points should be six millimeters apart. The Leyden jar should have a surface of 500 square centimeters, and give a spark one millimeter long. If, then, the discharges succeed each other at intervals of from three to five minutes, the following changes are observed in the coloured corpuscles of man. Firstly, the circular disks become slightly crenate. This effect gradually increases, the corpuscles become rosette-shaped, then mulberry-shaped, and finally, by the acumination of the projections, horse-chestnut shaped. Later, the processes are withdrawn, the blood corpuscle becomes round, and, at last, pale. In the corpuscles of the newt and frog the effects are not dissimilar. They become wrinkled and dappled, but these appearances are very transitory, and they are again seen to be circular and pale, while the nucleus becomes round and sharply defined. Not unfrequently it happens that one or more blood corpuscles coalesce before they lose their colour, or that (in amphibian blood) the nucleus is discharged while the disk is still yellow. The effects produced by *induction currents* are altogether analogous to those above described. Under the action of the *constant current* (a single Bunsen's cell) the corpuscles next the electrodes undergo changes, which at the negative pole correspond to the action of an acid, at the positive, to that of an alkali. In a salt preparation of batrachian blood examined near the positive pole, the nucleus comes first into view, and then the corpuscles lose their colour. In a similar preparation of human blood in which the corpuscles are horse-chestnut shaped already, they become smooth, lose their colour, and disappear.

The colourless corpuscles, when excited electrically during their amœboid movements, assume the spheroidal form. Their movements, however, are resumed as soon as the excitation is discontinued. The motion is more undulating than before, but soon recovers its former character. After repeated excitation the corpuscles expand into laminæ, but still exhibit changes of form. Under the influence of successive shocks of greater intensity, the colourless corpuscles swell out, their granules exhibiting molecular movement, and finally disappear.

Blood Crystals.—In concluding this chapter, we propose to give the most simple methods of obtaining crystals of hæmoglobin and hæmin for microscopic purposes, referring the reader for more detailed information to Chapter XIV.

Hæmoglobin.—A large drop of blood is taken directly from a living guineapig, and allowed to coagulate on a watch-glass. We now add a small quantity of water, and then, taking up the clot with the forceps, let fall on a glass slide several small drops. As these drops evaporate hæmoglobin crystals of varying size shoot out from the edge, separately and in bunches.

Another plan is to cut out the heart and great vessels of a recently killed guineapig, placing them on a watch-glass in saturated air for twenty-four hours. Then take some blood from the heart by means of a capillary tube, and allow a very small drop to fall into an equally small drop of water on a slide. As it evaporates, crystals are formed as before. This method does not answer with rabbit's blood.

Hæmin Crystals.—The simplest method of obtaining hæmin crystals is the following:—A small quantity of dried mammalian blood (human will do) is placed on a slide. A few small crystals of common salt are then added, and a cover-glass placed over. A drop of glacial acetic acid is then allowed to enter from the side. On warming the preparation carefully until the greater part of the acid has evaporated, an immense number of the reddish-brown crystals of hæmin are seen.

For a description of the corpuscles which occur in the lymphatic system, see the chapter treating of that subject. The development of the blood corpuscles will be described in Chapter VII.

CHAPTER II.

EPITHELIUM AND ENDOTHELIUM.

UNDER this heading are included the epithelium of the mucous membranes of the cornea and conjunctiva, and of the integument, and the endothelium of the serous membranes. The epithelium-like structures which are in relation with the nerves of the various organs of sense will be examined in Part II.

Ciliated Cylindrical Epithelium.—To investigate ciliated epithelium in the living state, a frog should be selected, and its mouth opened with the handle of a scalpel. Then, using either a lancet-shaped needle or the blade of a sharp knife, we scrape from the projection in the roof of the oral cavity, corresponding with the floor of the orbit, a little of its epithelial covering. This is transferred to a small drop of an indifferent fluid (half per cent. solution of common salt) on a glass slide, slightly separated with needles, and covered in the usual manner. In such a specimen we find not only masses of epithelium in connection, but also smaller groups and single cells. In the masses of epithelium we cannot distinguish quite clearly the individual cells, but on the free border—on the coast, as it were, of the epithelial island—we observe the exceedingly lively movement of the cilia. In addition we see blood disks, small round particles of protoplasm and granules driven quickly along in the fluid; and from these passing bodies we are able to recognize the direction of the movement of the cilia, an observation which could not otherwise be made, on account of the extreme rapidity of that movement. In the smaller epithelial groups we are able more easily to recognize the individual shortly-conical cells. These groups are in more or less rapid rotation, the rotatory motion being due to the fact that only one portion of their surface is furnished with cilia—that, namely, which corresponds to the bases of the conical cells.

Effects of Reagents on Ciliary Motion.—Dilute Alkalies.—After some time we perceive that the cilia here and there begin to strike more slowly, and, by-and-by, they come to rest. In a specimen prepared as above described, which has of course been prevented from becoming dry by the occasional addition of a drop of half per cent. solution of common salt, if we choose a spot at which the ciliary movement is either exceedingly languid or has ceased altogether, and cautiously allow a small quantity of a very delicate solution of potash to act upon it by the irrigation process, we soon observe that the motion is renewed; becoming equal in rapidity to that seen in the perfectly fresh preparation. The restoration of motion is not due to any special property of potash; nor can it be attributed to the influence of that reagent in dissolving coagulated material between the cilia, which might be supposed to interfere mechanically with their movements. This is proved by the fact that many other reagents act similarly as stimulants of ciliary motion—*e.g.*, distilled water, half per cent. solution of common salt, dilute acetic acid, carbonic acid, or the induced current (applied according to the method described in Chapter I.). All these, if used with great care, accelerate the movement in the first instance. The acceleration lasts only for a short time, and, in most cases, is quickly followed by cessation of movement, consequent upon the destructive influence of the reagent used. After the addition of *dilute acetic acid* (and still more rapidly with concentrated) the bodies of the cells swell and become transparent, and their nuclei well defined, in the same manner as after the addition of water. The investigation of the respective actions of *carbonic acid gas* and *oxygen* upon ciliary movement is a very important experiment. We make a preparation of the ciliated epithelium from the throat of the frog, in a half per cent. solution of common salt upon a cover-glass, which is then placed on a ring of putty over the gas-chamber of the movable stage (Fig. 2). Into this chamber a drop of water has been previously placed to keep it moist, and if we now allow a stream of carbonic acid to pass, we perceive, as has been already mentioned, that for a few moments the ciliary motion becomes quicker, but, by-and-by, slower, until it finally ceases. On now substituting atmospheric air (oxygen), we find that the movement slowly recommences, and, before long, is quite as active as before the passage of the carbonic acid. The experiment may be repeated several times with a like result, until at last the motion can no longer be excited. Oxygen is therefore as essential for the continuance of motion in the individual ciliated cell as for the maintenance of animal life in general.

Study of Ciliary Motion in Situ.—To demonstrate ciliary action on a membrane *in situ*, the most judicious plan is to remove from a female frog or toad that portion of peritoneum which covers the *cisterna lymphatica magna*, the so-called *septum* of the cisterna. Or, instead of this, a portion of the parietal peritoneum of the anterior abdominal wall of the newt may be employed. In either case, the part removed is to be quickly and carefully spread upon a glass slide with needles (avoiding every kind of mechanical injury) in such a manner that the peritoneal surface looks upwards: a drop of half per cent. solution of common salt is then placed on the under surface of the cover-glass, which is cautiously applied. In such a preparation we find places in which a bird's-eye view is obtained of the cilia in motion, as well as others, where—as in the preparation from the throat of the frog—we see the same in profile. The cells, which bear the cilia, are not cylindrical, but form a pavement endothelium, the elements of which are granular. We shall have occasion to return to these cells in the description of the endothelium of the septum. The *stomata* are almost always guarded by the cells above described. If we are uncertain of the direction in which the cilia strike, or if we wish to demonstrate this positively, we should transmit through the preparation, by the method of irrigation described in Chapter I., colouring matter, or some similar substance, in a finely divided state, such as ground animal charcoal, cinnabar, or Indian ink, suspended in half per cent. solution of common salt. We shall then be able to recognize, from the direction in which the particles are driven, the direction in which the cilia strike.

Forms of Ciliated Epithelium.—For the study of the various forms of ciliated cells, we remove a mucous membrane covered with these from a freshly-killed animal, and place small pieces of it in a sherry-coloured solution of bichromate of potash. After they have lain in the liquid for twenty-four hours or more, we scrape with a scalpel from the free surface a little of the epithelium,—place it on a slide in a small drop of bichromate of potash solution, or of common water, reduce it to fragments with the handle of a needle, and cover it. The most suitable objects for such a study are—the trachea of a mammal, the bell-shaped extremity of the Fallopian tube of the sow, and the mucous membrane of the mouth, throat, and œsophagus of the frog. By this mode of preparation the cells are preserved very perfectly. In the long conical cells with ciliated bases we have to notice the granular protoplasm which composes the body; the bright basal border; the sharply-defined ovoid nucleus, with its large single or double nucleolus; the long filamentous, simple or divided processes which penetrate between the cells of the deeper layers, and finally the cilia which pass out from the central protoplasm, perforating the basal border.

Besides these, we find intermediate forms of ciliated cells, which are shorter and broader, and which run out into one or two short, thick processes; and various forms of spindle-shaped cells, which, as we may convince ourselves, in large flakes of epithelium, wedge themselves, by means of processes of greater or less thickness, between the processes of the ciliated elements. They possess likewise an ovoid nucleus. Finally, there show themselves, here and there, long, conical cells (*goblet cells*), which, like the first-mentioned, run into a long process, and, in the thicker portion (Fig. 7*a*), are empty, or contain only a very few granules. The ampullate, or flask-shaped portion of these cells is bordered by a double-contoured membrane, which, at the basal end, is open, so that we have before us only the empty shell of the cell without the basal lid. Among a number of such cells swimming about, individuals occur, in which the open ends of the goblets can be seen, both obliquely and from the surface. In the deeper and thinner part of the cell, the protoplasm with the nucleus is, in most cases, still present, as represented in the figure. In a few examples part of the cell (Fig. 7*b*) is torn off, so that an empty funnel remains behind, in the extreme apex of which a small bit of protoplasm remains. If we look over a series of preparations, we shall certainly find examples in which the complete lid, or a portion of it, remains attached at one point only of the circumference, and floats freely otherwise. The appearances show that these goblet cells are nothing more than products of changes which have occurred in the ordinary conical ciliated cells. In the description of the epithelium of the intestine we shall again have an opportunity of referring to these cells.

Non-Ciliated Cylindrical Epithelium.—For the investigation of this form we use the epithelium of the papillæ of the tongue of the frog, and that of the intestinal canal of a mammal, either in the fresh condition or with the aid of reagents. From the dorsal surface of the frog's tongue a minute portion is snipped with curved scissors, transferred by means of a needle from the scissors on to a glass slide, and then, either covered without addition, the glass being pressed lightly down, or mounted in a drop of serum, or of half per cent. solution of common salt. The specimen must be examined with high powers (as, *e.g.*, Hartnack's No. 10 immersion). We see the numerous, thin, conical papillæ, both from above and in profile; the latter especially at the borders of the preparation. A papilla seen in profile exhibits on its surface a beautiful mosaic of pale cells, composed of finely granular protoplasm, marked off by sharp clear-shining lines of interstitial substance. If we fix our attention upon the borders and apices of the papillæ, we may convince ourselves that the mosaic is only the surface view of the conical or cylindrical cells, which cover and surround the papillæ.

Here and there we may easily perceive that these cells are coarsely granular, and that each contains a clear oval nucleus. Such coarsely-granular cells increase in number after the preparation has been mounted some time. We may mention that the cylindrical cells around the bases of the papillæ are generally ciliated.

Epithelium of Villi of Intestine.—In the rabbit we proceed as follows :—The animal is killed, the small intestine immediately opened, and from the borders (which then curl outwards) we remove a small portion with curved scissors as in the previous case. This is to be covered with the mucous surface upwards. The villi seen exhibit, on their surfaces, a regular mosaic of epithelium; at their borders, where the epithelium is in profile, it is seen to consist of regular cylindrical cells. If the observation of the mosaic is continued for some time, granular spherical bodies come into view; at first singly, but afterwards in numbers, which are raised above the general surface of the cells, as may be learnt by using the fine adjustment. These spherical bodies have escaped from the cylindrical cells. We shall see that it is by this means that the goblet cells already mentioned are produced. The epithelial cells on the borders of the villi display distinctly the broad, finely-striated border, which spreads over their ends like a cuticle. Equally instructive specimens may be obtained from the intestine of the cat, dog, guineapig, rat, or hedgehog. The epithelium of the villi may be as successfully studied, while still attached, in a preparation, mounted in serum, or half per cent. solution of common salt. For more prolonged examination, especially if we wish to study isolated cells, we put a piece of intestine, cut from the rabbit, dog, or cat, into a sherry-yellow solution of bichromate of potash, allow it to remain there for one or more days, and make our preparation in the manner already described with regard to the trachea. In such specimens we find not only numerous isolated cells, but also complete villi, and parts of the same, on which the epithelium, when its surface is viewed, resembles, as in the fresh preparation, a pavement of granular cells, each of which contains a relatively large, sharply-bordered, and apparently round nucleus. The lines of interstitial substance are sharp and dark. At the edges of each villus the epithelial cells are cylindrical, with finely-striated border. Each cell consists of granular protoplasm, and contains a sharply-defined nucleus, in which a distinct nucleolus is to be seen.

If we examine attentively the surface of a villus, or of a portion of villus (especially in a preparation from the intestine of the dog or cat, which has been allowed to remain in a solution of bichromate of potash), we shall find, between the mosaic of granular cells, roundish structures, either single or in small groups, and with a diameter greater than that of the cells of the mosaic; these are quite clear in the centre, have a doubly-contoured membrane, and give the impression of vesicular bodies. If we search on the borders of the villi for a structure in profile corresponding to this surface appearance, we find between the cylindrical cells, which are full of protoplasm, bodies of a bell- or goblet-shape, containing in the part which is next the tissue of the villus, a bit of protoplasm of variable size, refracting light strongly; within this is included a compressed, nuclear body. Amongst the isolated cells, also, we meet with numerous goblet-shaped ones, which may be examined in various positions. These cells are most numerous in the intestines of the dog and cat, in which it often occurs in preparations which have been kept in dilute chromic acid, or bichromate, that the epithelium is almost entirely transformed into goblet cells. The facts show that they are transformations of cylindrical epithelial cells, and that they may either be produced spontaneously, or, as more commonly happens, may be the product of certain reagents.

Pavement Epithelium.—This variety is well known to occur, chiefly as laminated epithelium, in the *conjunctiva corneæ, mucosa* of mouth and pharynx of mammals, and in the skin. In the urinary bladder of mammalia the epithelium is not purely pavement, but is mixed with, and shades off into, the cylindrical variety. We accordingly call it "transitional." The epithelium of the frog's urinary bladder is a single layer of pavement epithelium. That of the serous membranes, of the *membrana Descemeti*, and of the iris, consists mostly of a single layer of flat cells.

Fresh specimens of the *epithelium of the mouth* may be prepared either with indifferent reagents or with very dilute solution of bichromate of potash; but, if we wish to study the relation of the various layers of the laminated epithelium to each other, it is needful to make vertical sections through the superficial layers of the mucous membrane. To study the forms of the various cells of the separate layers, we may obtain a thin shred from the surface of the tongue or gums of a mammal by energetically scraping it with a scalpel. What is removed is broken up with needles, and covered either in half per cent. solution of common salt, or, what is quite as good, a very weak solution of bichromate of potash. In the surface layers of the epithelium, we find flat *tablet-shaped cells*, with small, oblong, strongly refracting nuclei; the borders of these cells are sharp and doubly-contoured. Their substance is mostly clear, containing only a few granules, generally situated in the immediate neighbourhood of the nucleus. Their surface is generally beset with irregular folds and furrows. If one of these cells is seen edgewise it appears spindle-shaped, because the thickness of the nucleus is greater than that of the cell. Besides these we find smaller polyhedric pavement cells, which consist of a nearly uniformly granular protoplasm, and possess one, or very rarely two, roundish, clear, and sharply-defined nuclei, with one or two large granules—*i.e.*, nucleoli—within them. Finally, if we have scraped very energetically with the scalpel, we meet with cells corresponding to the deepest layers, which possess more of a cylindrical form, and contain an oblong nucleus. Similar results may be obtained if we macerate a portion of the mucous membrane in bichromate of potash solution.

To study the *epithelium of the cornea* in the fresh condition we proceed in a somewhat similar way. A frog is held by an assistant, its nictitating membrane drawn down, and from the anterior corneal surface a thin layer is scraped with a lancet-shaped, or a cataract knife; the fragment removed is then broken up and covered in aqueous humour, or in half per cent. solution of common salt. Here we find not only isolated cells, but connected masses of epithelium arranged in layers. By means of the fine adjustment the individual cells of these layers may be studied; but we shall not at present occupy ourselves further either with the epithelium of the anterior corneal surface, or with the *membrana Descemeti,* since they will be fully described when we treat of the cornea.

The *epithelium of the skin* (epidermis), and especially of the elements of the *stratum corneum,* may be readily brought under investigation as follows:—A small shred is raised from either the back, or palm of the hand, and covered in water; reagents which act upon horny structures, as, *e.g.*, dilute and concentrated acids and alkalies, may then be added. For the study of the cells of the *Reta Malpighii,* or portion of the epidermis which lies upon the *corium,* or true skin, the pointed condylomata so frequently met with, are peculiarly suitable. Cancroid tumours are equally to be recommended. We place these structures in a sherry-coloured solution of bichromate of potash, and let them macerate there for several days. At the end of this time we scrape off a small portion of the epithelium with a scalpel, transfer it to a drop of water or bichromate solution on a slide, break it up with a needle-handle, and apply the cover-glass as usual. In such preparations we meet with very striking forms of the so-called ridged cells, *i.e.*, polyhedric cells whose surfaces are covered with ridges and intermediate furrows, and whose borders therefore, when seen in profile, appear as if serrated. Wherever two such surfaces are applied to each other, the ridges of the one fit into the furrows of the other, the line of adaptation being a zigzag one. The granular protoplasm of the individual cells, the sharply-bordered, ovoid, single or double nuclei, which sometimes lie in a vacuole in the protoplasm, and the nucleoli are clearly seen. Very interesting are the numerous *cells in various stages of division.* These are represented by the following forms:—1. Cells containing a single nucleus constricted into an hour-glass shape, with two nucleoli. 2. Cells which possess two nuclei lying quite close to each other, each with a nucleolus. 3. Cells with two nuclei lying at a distance from each other. Amongst those of the first form, some possess a shallow constriction; in some the constricting furrow is so deep, that the two portions of the cell are connected by a short bridge, which in others is reduced to a slender filament. The division of the nucleus is not always into two; it is not uncommon to find cells whose nucleus is rosette-shaped. Further, we meet with numerous large, flat cells, belonging to the most superficial layers, in whose interior is a vacuole of variable size, and shut up in this a young brood of from two or three, to eight or ten cells. This variety of proliferation is known as *endogenous.*

Epithelium of the Bladder.—As we have already remarked, the epithelium of the mucous membrane of the urinary bladder of mammals is laminated and transitional. A thin shred from the internal surface of the urinary bladder of the rabbit, guinea-pig, dog, or cat in the fresh state may be covered in half per cent. solution of common salt, in water, or in a bichromate solution. If the bladder has been kept from twenty-four to forty-eight hours in the latter liquid, specimens are obtained in which the following appearances may be observed:—Firstly, large pavement cells, bounded by a double contour, and consisting of a uniformly granular protoplasm which contains from two to five clear vesicular nuclei, each with a double contour, and possessing a large, shining nucleolus. In these pavement cells we see that, as a rule, only one of the surfaces is even; that, namely, which corresponds to the free surface of the mucous membrane. Of this we may convince ourselves by examination of connected masses of epithelium, or of vertical sections. The deep surface of each cell is marked by depressions with prominent ridges between them, and is that by which it is in contact with the club-shaped or conical cells of the subjacent layer, so that the rounded summits of the latter fit into the depressions of the former. The cells of the second layer consist of a uniformly granular protoplasm, have a double contour membrane, and each contains an oval vesicular nucleus, and within this a shining nucleolus. They possess simple or divided processes of varying length and thickness. Among them there are spindle-shaped cells, which insinuate themselves between the processes of the former layer.

To study the single layer of epithelium of the urinary bladder of the frog, consisting as it does of large granular cells, we spread upon a slide a portion of this organ with the free surface upwards, and cover it with a piece of thin glass, on the under surface of which a small drop of half per cent. solution of common salt has been placed. Wherever folds occur in the mucous membrane the epithelial cells show themselves in profile; where this is not the case, the surface view alone is obtained.

The Endothelium of the Serous Membranes.—The endothelium of the serous membranes, as well as that of the membranes related to them (for example, those which cover the posterior surface of the cornea and the iris of mammals, and the septa and walls of the lymph sacs of amphibia), is well known to consist of flat cells, the substance of which appears homogeneous when fresh, but becomes finely granular by the action of certain reagents. The nucleus is generally single, and occasions a projection of the free surface. It is usually oval and clear, and sometimes contains a nucleolus in its interior. Some cells contain two nuclei. By reason of the homogeneity of its protoplasm, the endothelium of the serous membranes is with difficulty brought into view in the fresh state. In folds, indeed, of a serous membrane which has been spread out upon a slide in a solution of common salt or in other indifferent reagents, the individual cells may be recognized in profile. Again, on the omentum, and on certain parts of the pleura of many animals, there occur bodies (which were first described by Sanderson as structures resembling lymph follicles, and which we shall describe at length in another place), the endothelium covering which may be seen in the fresh state to consist of granular cells which are polyhedral, but rounded on their free surfaces, each enclosing a rounded nucleus. On the fenestrated portion of the omentum, also, spots are met with where granular cells of the same form occur in groups, the elements of which appear to sprout out as it were from a common stem. Cells of the same kind are also found on the abdominal surface of the *centrum tendineum* of the diaphragm, over the structures to be afterwards described as *lymph channels*. Further, as we have already had occasion cursorily to remark, there occurs in the mesentery and parietal peritoneum—and, in the female of *Bufo* and *Rana*, on the septum separating the *cisterna lymphatica magna* from the peritoneal cavity—between the non-ciliated, homogeneous, large and flat endothelial cells, others which are ciliated, granular, small and polyhedral, occurring either singly or in groups. To bring these into view we have simply, as we have said, to remove a portion of the membrane in question from the recently killed animal, to spread it out carefully upon a slide with a couple of needles, avoiding all unnecessary dragging, and to cover it quickly before it becomes dry with a cover-glass on which a small drop of half per cent. solution of common salt, serum, or aqueous humour, has been placed.

The Silver Method.—The best method, however, and the one most frequently employed for exhibiting endothelium, is that of colouring by means of a solution of nitrate of silver. This method consists in bathing the fresh membrane, which, of course, has not been allowed to come into contact with blood or any injurious fluid, in a quarter or half per cent. solution of nitrate of silver. After immersion in this for a few minutes, it is washed out in ordinary water, which must be renewed as often as it becomes turbid, and is then exposed to the light until it assumes a brownish colour. The portion of membrane thus treated is spread out upon a glass slide and covered, a small drop of glycerine having been previously placed on the under surface of the cover-glass. On superficial examination a system of dark lines is seen, which bound clear spaces of various forms and sizes corresponding to the individual endothelial cells. Before mounting such a portion of membrane in glycerine, after having coloured it with silver, we may place it for a short time in very dilute ammoniacal carmine solution (to which, however, two small drops of acetic acid have been previously added), and then wash it in slightly acidulated water. We shall then find, on mounting the specimen, that nuclei appear in the spaces above mentioned: these are sometimes central, but more often to one side, and are oblong in form. According to the duration of the action of the carmine solution, and to its strength, they are more or less intensely coloured. By a modification of the silver method we may demonstrate, not only the nuclei and dark lines, but also the cell substance of the endothelia. This method always succeeds with the endothelium which lines the lymph sacs of the frog, and with that of the abdominal side of the diaphragm: sometimes also with the endothelium of other serous membranes. If we allow the membranes mentioned to lie for a longer time (ten to fifteen minutes) in a half per cent. nitrate of silver solution, and then simply wash them in water, and mount them in glycerine after they have acquired a brown colour, we shall be able to recognize, after an interval of from twelve to twenty-four hours, or often even earlier, the substance of the endothelial cells as a yellow or dark brown precipitation surrounding the clear oval nucleus. In preparing specimens with silver it is in general much to be recommended to mount the objects in glycerine, as soon as they have assumed a brownish tint, and not to leave them exposed to the light for an unnecessarily long time, otherwise they are apt to lose their beauty and clearness, from the occurrence of dark precipitates. In many parts of silver-coloured serous membranes a peculiar arrangement of the endothelium is observed, which consists in the existence of dark or clear spaces of various forms, around which the cells are set in a radiating manner. Each of these small apertures occurs at the point of junction of three or more endothelial cells, the interstitial lines of which radiate from the aperture. Such an arrangement we find on the portions of the abdominal side of the diaphragm, which correspond to the so-called lymph channels, on certain parts of the mesentery, and very abundantly in the pleura and omentum on the structures already mentioned as resembling lymph follicles. They are distinguished by the name of *stomata*, and are looked upon as the recipient openings of canals which belong to the lymphatic system. In the case of many of these cells this has not yet been proved; some of them have even been regarded as small endothelial portions of larger cells; while others give the impression of being accidental formations. Of such openings, or *stomata*, those that occur on the septum of the *cisterna lymphatica magna* of the frog may serve as the type. If we cut out this membrane from a frog or toad, spread it out and mount it in a solution of common salt, or in serum, or if instead we first colour it in silver and then mount it in glycerine, we shall find a proportionately large number of roundish or oblong openings between large radiating endothelial cells. These discontinuities represent the openings to short canals, which pass through the membranes and connect the abdominal cavity with that of the *cisterna lymphatica magna.* These openings are bordered by small granular cells, the convexities of which project into them. They are compactly arranged together, and each possesses a roundish nucleus. If the spreading out of the specimen has not been accomplished with sufficient care, or the membrane is too much shrunk, we miss the above-mentioned regular openings, and there appear instead only groups of small roundish cells—*i.e.*, the openings are collapsed, and the cells which line them have approached each other, so as to come in contact. The nature of the small bodies which project in the interior of the stomata has been disputed. It has been believed that they are nothing more than the nuclei of the large radiating endothelial cells which surround them. But, as we may convince ourselves both in fresh and in silver preparations, they are really endothelial cells seen in profile, which line the apertures. In female frogs and toads these cells are provided with cilia. In the chapter on lymphatic vessels we shall have an opportunity of making several additional remarks on the stomata.

We shall, in conclusion, endeavour to show that the lines which are brought out by nitrate of silver in the serous membranes are caused by precipitations for the most part in the albuminous substance which connects the cells, and not merely, as many authors believe, in an albuminous fluid which collects between their surfaces.. A serous membrane prepared from an animal just killed may be spread upon a cork plate and rinsed with one per cent. solution of sugar, or with a very dilute solution of glycerine, may even be brushed with a camel-hair pencil moistened with water (of course not too vigorously), without preventing the occurrence of the silver lines. Again, in a section prepared from a fresh mucous membrane, with laminated pavement epithelium, which section has been coloured in silver, the silver lines corresponding to the borders of the individual cells are distinguishable throughout all the layers. Further, silver lines corresponding with the borders of the individual muscle cells are met with in unstriped muscular tissue which has been coloured in silver, as, *e.g.*, in the muscular coats of arteries. These facts justify the assumption that the silver lines are caused by precipitations in the albuminous interstitial substance which bounds and separates the individual cells.

CHAPTER III.

CONNECTIVE TISSUES.

UNDER this heading we include the fibrous tissues, with the cellular elements which they contain, the elastic tissues, cartilage, and bone.

Fibrous Tissue.—Fibrous tissue consists of delicate gelatigenous fibres, which are connected by an interstitial albuminous substance. The fibres form bundles of various thickness, which either have a parallel arrangement, as in tendons and fasciæ; or form a meshwork by the splitting and reunion of neighbouring bundles, as in the omentum, the submucous and subcutaneous tissue; or, finally, have a felt-like arrangement, in which the bundles cross each other, or twist round one another in the most complicated manner, as in the skin and mucous membranes. Fibrous tissue may be studied in the fresh state, or after maceration, or in hardened preparations. To examine the tissue in the fresh state it is best to make a preparation of a tendon by teasing. A small tendon (such as, *e.g.*, one of the extensors of the toes) having been cut out from a recently killed frog or rabbit, is placed from ten to fifteen minutes in a five to ten per cent. solution of chloride of sodium, whereby the splitting of the tendon is considerably facilitated.

Process of Teasing.—In making preparations by teasing, the following practical rules must be attended to:—A very small portion must be used; this must be placed on the glass in a drop of the liquid to be employed, which must also be small, for if in too great quantity the particles teased out, swim away in the liquid, and are difficult to seize upon with the needle. On the other hand care must be taken, as the liquid evaporates, to add more, so as not to allow the preparation to become dry. In the preparation of tissues which consist of several parallel bundles, such as nerves, tendons, or muscular tissue, our object is to divide the fragments in the direction of the fibres into smaller and smaller portions. Even when the tissue consists of elements which tend in no particular direction, it is still desirable to follow one direction in teasing—the object being best attained by first fixing the fragment with one needle, then piercing it with the other held in the opposite direction, and finally drawing the two apart. It is further noteworthy that the teasing must be performed on the centre of a slide, and limited within an area which is not larger than the cover-glass. The drop of fluid in which the preparation is to be mounted should be placed on the cover-glass, which must then be inverted upon it. As the liquid evaporates it must be renewed from time to time.

Action of Acetic Acid on Fibrous Tissues.—In a teased preparation of tendon in salt solution, bundles of very fine homogeneous-looking fibres are seen. If the preparation is irrigated with weak acetic acid the bundles are seen to swell out, become homogeneous, and completely disappear. If concentrated acid is used the effect is more rapid.

Areolar Tissue.—In a portion of fresh mesentery (of a frog or of a small mammalian animal) spread out on a glass slide and mounted in salt solution, we have the shining wavy bundles forming a felt-work. In the omentum or pleura of a guineapig or of a cat, prepared in a similar way, the arrangement is that of a meshwork. From each larger bundle we see several smaller ones splitting off, and then meeting with similar ones which are branches of other larger bundles in the neighbourhood. (*See* Fig. 8). According to the abundance of these collateral or secondary bundles, and the way in which they run, the meshes vary in size and form, being round, rhombic, or oblong.

Effect of Maceration.—For the purpose of macerating fibrous tissue, ten per cent. solution of common salt, lime water, baryta water, or solution of permanganate of potash may be used. By all these reagents the interstitial albuminous substance is dissolved out, so that the bundles split into their constituent fibres. All that is then necessary to display them is to prepare a small fragment with needles. Diluted bichromate of potash solution may also be used, but its action is very slow.

Elastic Tissue.—Elastic tissue is characterized specially by the facts that the elementary fibres of which it consists do not swell in acids, that they do not yield gelatin in boiling, and that in general they are not united into bundles, but occur as sharply defined threads which run an isolated course, sometimes straight, sometimes contorted, or even spiral. By repeated bifurcations and fusions of the branches again with one another, they form a network. These facts may be demonstrated very advantageously in a serous membrane, particularly in the mesocolon of the rabbit, or in that part of the parietal peritoneum of the same animal which lies on either side of the lumbar vertebræ. In both of these situations the elastic fibres are very strongly developed. If preparations of these or similar membranes are treated with acetic acid, the bundles of common connective tissue disappear, so that the network of elastic fibres becomes prominent.

To show the elastic fibres of the *ligamentum nuchæ,* the best way is to make preparations by teasing a portion of that of the ox, in salt solution, either in the fresh condition, or after maceration for a day or more in sherry-coloured solution of bichromate of potash. In either case we have before us thick, solid, shiny cords of homogeneous substance, which branch dichotomously, uniting by their branches so as to form a network. The individual fibres, however, run mostly in one direction, and are so close to one another, that, on superficial examination, they exhibit the appearance of a reticular arrangement. Such fibres as happen to be separated from the rest are often rolled up like a watch-spring.

The dichotomously-branching *elastic fibres of the pulmonary substance* can be shown, either by teasing fragments of fresh lung (an operation which requires an immense deal of patience), or in sections of fresh lung hardened by freezing, as will be afterwards described. The elastic so-called *fenestrated membranes* which exist in the *tunica intima* of the large arteries may be demonstrated as follows :—A part of the aorta of a rabbit or guineapig having been cut out,is pinned down on a flat cork with the internal surface upwards. The membrane having been fixed at a certain point with a needle, the *intima* is raised up close to the latter with sharp forceps, and then shreds as long as possible are stripped off—a process which requires no remarkable skill. Any one possessed of the requisite dexterity may then strip off thin lamellæ from the deep surface of these shreds; these may be at once mounted, and are so thin that the fenestrated membrane can be seen at the edges without further preparation. If this does not succeed, the student must content himself with teasing out the shreds first obtained.

Finally, a network of elastic fibres can be shown very beautifully in the vocal cords of the frog. To any one who is sufficiently acquainted with the general anatomical relations of the parts, it is not difficult to remove those structures even from the living animal. The easiest way is to place the vocal cord for a few minutes in dilute acetic acid, and then to scrape off the epithelium with a lancet-shaped needle—a process which is much facilitated by the previous steeping in the acid. The preparation is then mounted in glycerin.

Cellular Elements of the Connective Tissue.—These are either amœboid—*i.e.*, migratory cells; or branched—*i.e.*, fixed cells; the latter being distinguished further by the union of their branched or simple processes, so as to form networks of various densities.

Amœboid Cells.—These are to be found in every form of connective tissue. Normally, they occur only in small numbers, and are irregularly distributed; but, in inflammation, they are numerous in proportion to the intensity of the process, their multiplication being sometimes scarcely observable, while, at other times, they are so numerous as to fill up the tissue. Two kinds may be distinguished: the cells of the first form entirely resemble the colourless blood corpuscles—*i.e.*, they consist of finely granular protoplasm, contain two or more nuclei, exhibit amœboid movements, and are similarly affected by reagents; while those of the second form are large, coarsely granular cells, which, like the granular cells of the blood, are characterized by the rounded contour of their processes. The former are to be found in every connective tissue, but the latter are more common in the subcutaneous and submucous tissue, in the intermuscular connective tissue, in the mesentery, in the neighbourhood of blood vessels, in the septa of the subcutaneous lymph sacs of the frog or toad, and in the neurilemma of the larger nerve trunks of the frog. The two forms graduate into each other.

The method of studying these cells in the living condition consists simply in spreading out thin shreds of connective tissue on a glass slide, and mounting them in indifferent liquids. Where the integument is loose, as in the neck of mammalia, &c., it is easy to effect this, by first making a slit in the skin, and then, with curved scissors, snipping away a thin lamella of subcutaneous tissue. In the frog, the tongue may be drawn out and fixed by an assistant, while the operator snips out a portion, so as to obtain a cut-surface, from which a thin lamella can be readily taken, as above. In either case the lamella must be spread out, without loss of time, and with as little displacement as possible, on the slide, and mounted in *humor aqueus* or fresh serum. Blood corpuscles which exist on the surface of the preparation do not interfere with the object, because the amœboid cells are to be found in the interstices of the clear transparent fibrillated mass of fibrous tissue.—It is somewhat more difficult to demonstrate the *migratory cells* of the normal *cornea*. The method is as follows:—A frog is held by an assistant in such a way that the *bulbus oculi* is tense. The *membrana nictitans* is then drawn back, and the bulb penetrated with a cataract knife, just as in the operation for cataract, at the *limbus conjunctivæ* next the inner *canthus*. The point of the knife is advanced until it approaches the limbus of the opposite side, without puncturing it, and is then carried outwards and upwards, so as to form a flap, consisting of the upper half of the cornea. The extreme edge of the flap must then be seized with the forceps, while the lower half of the cornea is cut away with the aid of scissors curved in the direction of their edge. The cornea is next transferred to a drop of *humor aqueus* (previously obtained by puncturing the opposite eye) and spread out on the glass slide with the anterior surface uppermost. In order to avoid folds, it is desirable to make two or three radial incisions. The preparation is now covered and enclosed in oil. If it is desired to study the migratory cells on the warm stage, the preparation must of course be mounted between two cover-glasses, as before directed.

If a cornea is thus prepared with great care, nothing is to be seen excepting that a few pale lines of interstitial substance, referable to the anterior epithelium, may be distinguished where the membrane is folded. No other optical differences can be made out. If the individual epithelial elements can be distinguished, this affords proof that the object has been injured in preparation. Notwithstanding its homogeneity, it is possible (with the No. 10 immersion objective of Hartnack) to find out the upper and under surfaces of the cornea by means of coloured blood corpuscles, pigment granules, or retina-elements which may happen to be in contact with them. As time goes on, the interstitial lines of the anterior epithelium come into view. If we then adjust the microscope so as to bring into view the most superficial layer of the propria, a few corpuscles of more or less irregular form can be detected, each of which consists of almost hyaline protoplasm, and contains a nucleus of irregular form, apparently finely granular. If one of these corpuscles is watched carefully, it is seen that changes of form take place, both in the protoplasm and in the nucleus. The corpuscles throw out processes and retract them, and even perform a certain amount of locomotion. The nuclei become constricted or compressed, and again resume their original form, to undergo similar changes. By and by similar corpuscles become visible in the depth of the cornea. On the warm stage the movements are naturally more active. (*See* Chapter XIV.)

If a preparation is made in *humor aqueus* of the fresh peritoneum (particularly the omentum) of the frog or of a mammal, or of a septum of a subcutaneous lymph sac of the former, innumerable migratory cells are seen, especially in the neighbourhood of the vessels, which present transitions between small pale corpuscles and large granular ones, all exhibiting distinct amœboid movements. But the best place for observing these bodies is the tail of the tadpole. If a portion of the tail is taken from the thin membranous part, and mounted in half per cent. salt solution, migratory cells are to be found everywhere, consisting of finely granular protoplasm, and displaying extremely active movements.

With reference to the *granular corpuscles* it is not necessary to add much to what has already been said. Among the best examples are certain coarsely granular elements, which occur in the intermuscular connective tissue of the frog. If the transparent membrane which separates the muscles of the thigh of the frog is spread out and examined in an indifferent liquid, it is found that, besides active migratory cells, there are coarsely granular elements possessing oblong nuclei of the most various forms, which move very sluggishly. Perfectly similar bodies occur in the sheaths of large nerves of the frog. Another situation for studying these cells in great numbers is the tongue of the same animal. A living frog having been secured in the supine position, its mouth is opened and the tongue is drawn out by its two cornua. Thin shreds are then snipped from the substance of the organ (the epithelium having been first removed in the same way) and covered in fresh serum. In a preparation thus made, an immense number of large coarsely granular cells appear, presenting the most grotesque forms. (*See* Fig. 9.)

Branched Cells (Connective Tissue Corpuscles).—These bodies are flattened cells, consisting of finely granular protoplasm: each contains a nucleus, which is also, for the most part, flattened and oblong. They possess a greater or less number of processes; and by these, which are sometimes branched, sometimes single, they are in continuity with each other, so as to form a network. In some connective tissues the processes exhibit a more or less regular relation to the body of the corpuscles; in others, they are so short that the corpuscles are almost in contact with each other, being separated by scarcely any interstitial substance. In preparations made in the way already recommended for the demonstration of amœboid cells of the subcutaneous connective tissue of the rabbit, bodies are also found which are distinguished from the others by their very irregular placoid form, greater size, and hyaline appearance, as well as by the possession of oblong nuclei. These cells contain very few granules, and those mostly in the neighbourhood of the nucleus. At first sight these placoids seem to have only short projections, but, under high powers, they are found to possess numerous long hyaline radiating processes.

Fixed Corpuscles of the Cornea.—In a cornea prepared in the manner previously described, it is possible to recognize the network of pale branched corpuscles at all depths, after some time has elapsed. They may, however, be more distinctly shown with the aid of certain reagents, particularly wood vinegar, nitrate of silver, chloride of gold, and some other metallic salts. Of these, the first is now laid aside in favour of the others. In preparations obtained by stripping off shreds of a cornea (of the rabbit or frog), which has been macerated for twenty-four hours in wood vinegar, the corpuscles are seen as large flattened cells, consisting of granulous protoplasm, communicating with one another by processes. If vertical sections are made of such a cornea, the cells seem to be spindle-shaped; but, if the section is made obliquely, it is found that the corpuscles appear the more flattened and the more branched, the greater the obliquity of the section. This fact proves that the corpuscles are flattened in planes parallel to the surface, and that the processes also stretch out in similar planes.

Treatment of the Cornea with Nitrate of Silver.—Nitrate of silver is used both in substance and in solution. In substance it may be employed in two ways:—*a.* The centre of the cornea of a frog, which is held by an assistant in the manner previously described, is firmly cauterized with a pointed stick of lunar caustic. One or two drops of salt solution are then allowed to flow over the cornea to decompose the excess of nitrate of silver. About an hour after the cauterization, the cornea is excised in the manner directed in p. 35, washed in water for several minutes, and the surface of the slough cleansed by pencilling it lightly under water. In the case of the frog's cornea, the central cauterized part may be cut out and mounted in glycerin at once; but the rabbit's cornea is so thick that it is necessary to split it into layers, with the help of fine pointed forceps. If the preparation has been exposed to daylight, clear spaces are seen on a brown, yellow, or dark ground, which communicate with one another by clear channels, either branched or single. These correspond in form and configuration with the network of corpuscles above described. This signifies that we have before us, as will be more completely shown afterwards, *the spaces which the corpuscles occupy.* This network of clear spaces represents the canalicular system (*Saftcanälchen System*) of the cornea: it must not be confused with Bowman's tubes.—*b.* The second method of applying the nitrate of silver in substance has the advantage that it shows the canalicular system in all parts of the cornea. It consists in first scraping the cornea of a living frog or small mammal with a sharp cataract knife, so as to remove the epithelium completely. After a little practice, and provided the bulb is properly fixed by an assistant, it is not difficult to perform this operation without injuring the substance of the cornea. Thereupon the caustic is two or three times lightly rubbed over the whole surface, after which the eye is washed with saline solution, and the animal is left to itself for twenty or thirty minutes. The cornea is then excised, washed in ordinary water for several minutes, and pencilled with a camel-hair brush. The mode of preparation is as before, care being taken to make one or two radial incisions, in order that the membrane may lie flat on the glass surface. After the preparation has been exposed for a few hours, the contrast between the spaces and the yellowish-brown interstitial substance becomes very obvious.

[The endothelium of Descemet's membrane, with its dark interstitial lines, brownish-yellow cell substance, and clear ovoid or lobed nuclei, is well seen. It is to be noted that all preparations of this kind must be kept in the dark.]

Similar results are obtained by the use of the nitrate of silver in solution. With this view the epithelium is either pencilled off from the anterior surface with warm water, or scraped off as above described. The cornea is then immediately excised and immersed for fifteen or twenty minutes in a half to one per cent. solution. It is then washed and prepared as above. If, however, after washing the preparation for a very short time, it is transferred to a ten per cent. solution of chloride of sodium for five or ten minutes, and is then again washed in ordinary water and mounted in glycerin, the appearance is very different. We have before us in most parts the canalicular system marked out by a dark precipitate, while the interstitial substance remains almost clear. In other parts there are gradations of staining between this appearance and the negative staining obtained by the ordinary method.

Preparation of the Cornea with Chloride of Gold.—The fresh cornea of a frog or mammal is placed in as much half per cent. solution of pure chloride of gold as is sufficient to cover it, and left immersed until it acquires a straw-yellow colour—*i.e.*, at most thirty minutes. Thereupon it is transferred to distilled water, or water slightly acidulated. The preparation passes through pale grey, then dark grey, violet grey, violet and reddish, to dark red—the time required for the production of the last-mentioned colour differing, *cæteris paribus*, according to the time during which it was immersed, and the intensity of the light. In the height of summer, twenty-four hours, or even less time, is sufficient; but in winter several days are required, in which case it is preferable to use distilled, rather than acidulated, water, because the latter is apt to produce too much swelling of the preparation. From a darkly coloured cornea so prepared, the anterior epithelium is removed by stripping it off from the *annulus conjunctivæ* inwards, with the aid of a sharp pointed forceps. If that of a frog, the cornea may then be mounted in glycerin without further preparation. The rabbit's cornea must be prepared as before directed. In this way;

one of the most beautiful preparations in the whole range of histology is obtained. The bodies and processes of the corpuscles are seen to consist of a more or less granular protoplasm of various shades of violet. Each corpuscle contains a flattened oblong, well-defined nucleus, which is of a violet colour, and encloses one or two large, round, dark-coloured nucleoli. (Fig. 10.) Corneas stained with chloride of gold may also be advantageously studied by vertical sections, and by sections parallel with the surface: from such sections it is easy for any one to satisfy himself that the structures seen actually exist as such, and are not the products of the mode of preparation. It is, however, necessary to demonstrate that the canalicular network which we see with such distinctness in silver preparations, corresponds to and coincides with the network of branched corpuscles displayed in gold preparations, in such a way as to make it certain that the latter fit into and fill out the former. There are two modes of proof:—*a.* A frog's cornea is prepared and mounted, *lege artis,* on the glass slide (Fig. 6), and is then examined with a No. 10 immersion, objective, while an induced current of moderate strength is caused to act upon it. After the excitation, the system of branched corpuscles becomes distinguishable, and each is seen to be surrounded with a clear margin. After a time this appearance is lost, but can be reproduced by repeating the excitation. It admits of but one interpretation, viz., that the protoplasm contracts, under the excitation, in such a way as no longer to fill out the space in which it is contained—again occupying it as soon as the contraction ceases. *b.* A rabbit's cornea is gently rubbed with caustic until the epithelium is removed as a slough. After from twenty to thirty minutes a few drops of concentrated solution of chloride of gold are placed on the cornea. The eye is left to itself for fifteen or twenty minutes, after which time the cornea is shaved off with a razor, and steeped for twenty-four hours in water feebly acidulated with acetic acid. It is then not difficult to prepare from the parchment-like cornea, with sharp forceps, thin lamellæ; or to make thin sections, in planes parallel with the surface, with a razor. In preparations of either kind mounted in glycerin, even when examined with the naked eye, three different colours may be distinguished. There are patches of grey and others of violet; and these two are separated from each other by intermediate regions of a dull violet-red. Under the microscope the grey parts exhibit the characteristic appearance of silver preparations—a clear canalicular system on a yellowish-brown interstitial substance. In the violet parts the canalicular system is also clear, but the interstitial substance is violet; whereas, in the dull red parts, there are bluish or dull red corpuscles on a clear ground. Both in the first and in the second, there are transitions to the intermediate parts, *i.e.*, the nearer the part observed is to the edge of the dull violet-red parts, the more possible is it to make out that the network of protoplasm occupies the canalicular system. It is always possible to find points where the processes of protoplasm stretch from these parts into clear canaliculi.

Branched Corpuscles of the Tail of the Tadpole.—Another object in which it is easy to demonstrate the branched corpuscles, is the tail of the tadpole. In this organ, when prepared in the fresh state, as above directed, a very beautiful network of pale protoplasm, in a hyaline interstitial substance, may be demonstrated. The network consists of nucleated cells, which communicate with one another by dendritic processes. It is most dense near the edges and toward the tip of the tail. In order to obtain preparations of this structure, it is best to place a portion of the organ of a tadpole (in which the posterior extremities have begun to sprout) in half per cent. solution of chloride of gold for from thirty to forty minutes. The preparation having been placed for twenty-four hours in distilled water, and exposed to light, the epithelium of one side must be removed. For this purpose the organ must be fixed by a needle in the middle line close to the cut end : the epithelium, with the plexus of nerves and blood-vessels of one side, can be stripped off, with the fine-pointed forceps, in the form of a membrane—a process which is much facilitated by first placing the preparation for fifteen minutes in absolute alcohol. The separated structures are then covered in glycerin. Such preparations are of great value, serving not merely for the demonstration of the cells with which we are now concerned, but also, as will be seen, for the study of the structure and development of the capillary blood-vessels, of the most minute nerve fibres, and the relation of the lymphatic vessels to the connective tissue elements.—The description and mode of demonstration of the branched cells of the serous membranes will be given in the chapter on the lymphatic system, in connection with which they are of most importance.

Branched Corpuscles of the Skin.—In order to demonstrate the branched cells of the cutis (or of the mucosa), it is best to snip off folds or ribands from the fresh structure with the curved scissors. These are placed in half per cent. solution of chloride of gold until they acquire a distinctly yellow tinge. They are then transferred into distilled water until they are tinged dark violet and finally hardened in ordinary alcohol. Sections must then be made parallel to the surface, and covered in glycerin. Sections in this direction are preferable, because the branching of the cells and their mode of communication cannot be so well seen in others. We shall return to these subsequently. In the *membrana nictitans* of the frog there occur networks of large coarsely granulated cells, containing flattened oblong nuclei, and with branches which run for the most part parallel with the surface. This structure must be prepared with chloride of gold, in exactly the same way as the cornea.

Pigment Cells.—These are closely related to the fixed cells now under consideration. They are more or less branched corpuscles, which are sometimes isolated, sometimes form a network. They are, in general, larger than the ordinary connective tissue corpuscles. Each contains an oblong clear nucleus, while both their bodies and processes are beset with pigment granules. In mammalia they are found, as is well known, especially in the skin, and in the sclerotic, iris, and choroid. In the lower vertebrates, *e.g.*, in the frog, they are very numerous, not only in the skin, but in the peritoneum, and in several mucous membranes. Pigment cells can be made to retract their pigmented processes, when stimulated either mechanically, chemically, or electrically, as well as under the influence of light. Let us examine them (*a*) in the web, (*b*) in the mesentery of the frog, (*c*) in the tail of the tadpole, and (*d*) in the choroid of a mammal. (*a*) A common frog (*R. temporaria*) is secured on a plate similar to that shown in Fig. 11, and the toes are extended by ligatures attached to their tips. With this view, the hole O is surrounded by five or six small perforations, into which wooden pins can be stuck; the ends of the ligatures are drawn through the holes and fastened with the pins. In those parts of the web, which appear to the naked eye dark, it is seen, even with a linear magnification of 100, that the pigment cells are connected by an extraordinary number of fine dark processes, which are either penicilliated or dendritic. Often the distinction between body and process is not marked; it looks rather as if the whole network were made up of processes. In other parts, which are not so dark to the naked eye, groups of pigment cells are found, in which the bodies are round or oblong, and the processes broader and less numerous—the latter being either in continuity with those of neighbouring corpuscles, or broken off abruptly by a gnawed edge. The pigment granules do not extend to the end of these broad processes; so that it is possible to see that the substance in which they are embedded is hyaline.

If the dark parts are touched once or twice with a camel-hair pencil (especially if it has been dipped in oil of turpentine), the processes are gradually retracted, while, *pari passu*, the skin becomes visibly paler. On resuming the observation, after the lapse of one or two hours, it is found that the pigmented network is as dense, and the processes are as numerous, as at the beginning of the observation. It is a remarkable fact that the projection of the processes is much accelerated by the application of a drop of croton oil, with the aid of a capillary pipette, to the irritated part. In certain places, where the cells are not entirely black, but have a more or less yellowish-brown colour, and possess only a few stumpy processes, these last undergo spontaneous changes of form, as regards length and thickness. When the web is irritated, these, like the others, retract their processes altogether. If the circulation is arrested by placing a ligature around the leg, the pigment cells on the same side acquire a brighter colour—the dull brownish-yellow tint returning with the restoration of the circulation.

In the tail of the tadpole the pigment cells in several respects resemble the ordinary branched cells. The most superficial extend themselves by their processes between the epithelial cells. In the tadpole of the toad, which is distinguished from that of the frog by the breadth and shortness of the tail, they are spindle-shaped, and form by their processes a tolerably regular lattice-work, with nearly rectangular spaces, which is uniformly distributed throughout the tissue; immediately underneath the epithelium, however, there are some cells, the mode of branching of which is dendritic. In fresh preparations, or in preparations with chloride of gold, of the mesentery of the frog, a greater or less number of pigment cells are seen in the immediate neighbourhood of the large blood-vessels, and especially the arteries, and often form a complete sheath around them. Isolated pigment cells occur also elsewhere in the tissue. With high powers (No 10 immersion) and with dilute acetic acid, it is possible to make out in fresh preparations of the nictitating membrane and mesentery that the whole cell is not pigmented, the pigment being confined to certain parts of the body and to the axes of some of the processes. In mammalia, the most varied forms of pigment cells occur in the choroid and sclerotic, from the irregularly formed cells with slight knob-shaped projections containing coarse pigment granules, to cells with regular dendritic branching and fine granules.

Fat Cells.—Fat cells are distinguished from ordinary branched connective tissue corpuscles mainly by the fact that they contain drops of fat. When an ordinary branched cell undergoes conversion into a fat cell, the change commences by the appearance of small droplets in the protoplasm. By the confluence of these with each other a larger drop is formed. As this increases, the protoplasm of the corpuscles is distended more and more, until it forms around the globule a thin investment, in which lies the clear oblong nucleus. In well-developed fat cells, which usually lie together in groups, it is not possible to observe processes. They rather resemble closely packed globular structures.

Transition Forms between Connective Tissue Corpuscles and Fat Cells.—If, in a rabbit, the skin and subcutaneous tissue are divided over the inner (anterior) third of the infra-orbital edge, and the thin membrane which stretches over the infra-orbital fossa is severed, it is easy to remove, along with the *glandula infraorbitalis*, a gelatinous hyaline mass. If, from this mass, a very thin portion is snipped off and placed in a drop of fresh serum on a glass side and covered, it is easy to distinguish, among the ordinary branched cells, others which are larger and contain globules of fat. All transitions may be seen between those which contain one or two small droplets and those which are completely distended. These structures will be referred to again, under another heading. Fat cells are, as a rule, collected in masses around blood-vessels.

Tendon Cells.—The cells of mature tèndon tissue do not essentially differ from those of ordinary connective tissue. Like them, they are oblate branched masses of protoplasm, which are in communication with one another by their processes. They are not, however, flat, but curve themselves in conformity with the surfaces of the individual bundles to which they are applied. In order to study them, the best material is afforded by the tail-tendons both of young and full-grown rats or of rabbits, which can be examined either in the fresh state in serum, or by steeping them for a few minutes in silver solution, after they have been first pencilled with a camel-hair brush dipped in fresh serum.—Another material which may be used is the *centrum tendineum* of the diaphragm. In very young animals the caudal tendons present a peculiar arrangement. If the tail of a very young rat is amputated, and the tip torn asunder from the cut end, a great number of isolated lengths of tendon are obtained, of almost microscopic tenuity. These may be at once separated, and covered in very dilute acetic acid. Such a preparation shows, between the individual bundles, chains of apparently quadrangular masses of protoplasm, each containing a roundish nucleus. These chains alternate in position with the bundles. If, however, a single cylindrical bundle of fibrils is separated, it is seen that it possesses an envelope of granulous protoplasm, which extends along one side of the bundle, covering nearly half of its circumference; in this envelope nuclei lie arranged in linear series.—If the preparations are treated with stronger acetic acid, the protoplasm between the nuclei exhibits cross lines of interstitial substance. Hence it is evident that the sheath of protoplasm with which nearly the half of each individual bundle is surrounded consists of a series of hollow half-cylinders with their ends in apposition. To preserve the preparations above referred to, the fresh tendon should be placed for a very short time in acidulated water, until it begins to swell just perceptibly; it is then to be transferred to half per cent. solution of chloride of gold for ten or fifteen minutes, and washed in distilled water till it acquires a rich colour, and then to be mounted in glycerin. In cross sections through young tendons of the rat or rabbit, in consequence of the anatomical facts already stated, the bundles look as if they were contained in the meshes of a network of protoplasm, with nuclei at the nodes. Such sections may be best prepared from the caudal tendons, or from the *T. Achilles* treated with gold and then hardened in common alcohol.

Adenoid Tissue.—It remains to describe the so-called adenoid tissue. By this term is understood a dense reticulum of branched cells, the processes of which are short but of great delicacy. The younger the individual, the more the material of which the reticulum is composed possesses the character of protoplasm; the older, the more homogeneous the processes appear, and the smaller the quantity of protoplasm at the nodes, which correspond to the bodies of the cells. There are great differences between the several forms of adenoid tissue, which it will be most advantageous to study in connection with the tissues in which they are respectively met with, *e.g.*, lymphatic glands, intestinal mucosa, &c. The best objects for study are the mesenteric glands or the thymus of the calf, and the lymphatic follicle of the intestine of the rabbit. These must be hardened in Müller's fluid or in diluted alcohol. As soon as the tissue has become firm enough, thin sections are prepared, which are agitated with water in a test tube, until they present the appearance of a reticular membrane. They are then covered in glycerin, with or without previous staining. For more minute descriptions, see Chapter VI.

Development of Connective Tissue.—Fibrous connective tissue is developed from cells in two ways, as follows:—

At a certain stage of embryonal life, those organs which, at birth and in the adult consist of fibrous tissue, are composed exclusively of embryonal cells. As development proceeds, these cells, which are originally roundish, are either transformed into a network of branched cells, or lengthened out, so as to form bundles of spindle-shaped cells. At first both the bodies and processes of the cells, whether branched or spindle-shaped, consist of granulous protoplasm. The protoplasm subsequently undergoes a process of splitting, by which it is transformed into fibrils. This change commences in the processes, progressing towards the body. The fibrils are united to one another by an interfibrillar substance, which, at first granular, afterwards becomes homogeneous. In this way the network of branched cells is transformed into fine fibres, arranged in a meshwork; while the spindle-shaped cells are converted into collections of fibres, running in parallel bundles. A third mode consists in the transformation of isolated spindle or branched cells, which, according to the number of their simple or divided processes, split into a corresponding number of bundles of fibres. For the study of the process it is best to employ the umbilical cord, the skin, tendons, or the mucous membrane of the mouth or bladder of young embryos of man or animals. The parts in question should be kept for some time in sherry-yellow solution of bichromate of potash, after which the tissues may be prepared by teasing in a drop of the solution or in water. It is also desirable, especially as regards tendons, skin, and mucous membrane of the bladder, to make thin sections, after hardening in solutions of chromic acid or chromates.—A splendid object is to be found in the abundant gelatinous substance which covers the internal surface of the gravid uterus of the sow, and extends from thence over the external surface of the membranes of the ovum. If a very small portion of this substance is placed in salt solution on a glass slide, and covered without any further preparation, very remarkable forms of large branched or spindle-shaped cells are seen, which consist of evenly granular protoplasm, and contain roundish or oblong sharply defined nuclei. The branches are often so large as to stretch over the whole field (No. 8 Hartnack), and they may be seen to split out at their ends into sheaves of the most delicate fibrils. From the abundant submucous spongy tissue of the gravid uterus of the same animal, instructive preparations may be obtained (by stripping off fine portions with the curved scissors), which merely require to be spread out with needles in salt solution. If the preparation is to be kept, it may be placed in bichromate of potash and afterwards transferred to glycerin. In the gelatinous substance previously described as found in the infra-orbital fossa of the rabbit, isolated delicate wavy bundles of connective tissue occur, which, after a shorter or longer course, is seen to be in close relation with processes of slender pale cells which contain round nuclei.

Hyaline Cartilage.—For the study of hyaline cartilage, the episternum of the frog and the thin expansion of the shoulder-girdle of the newt are good objects. If the thin part of either of these is prepared in half per cent. saline solution or in serum, after the perichondrium has been carefully separated with the aid of the sharp forceps, the oblong or spherical cartilage cells are seen embedded in a hyaline or finely granular matrix. The edges of the cells are sharply defined, their substance is clear, or beset with a very few granules, their nuclei are also somewhat granulous. At all depths the intercellular substance (ground substance or matrix) can be seen, under high powers, to be divided into territories, each corresponding to a cell. So long as the preparation is fresh, most of the cells completely fill the cavities which they occupy in the matrix; these cavities are termed capsules. Only here and there can a clear space be distinguished between the external surface of the cell and the wall of the capsule. For the most part each cell contains a single nucleus; there are, however, some which contain two nuclei. In the middle part of the preparation, they are found either singly and at equal distances from each other, or in pairs, *i.e.*, two in one capsule, united by straight lines of contact. Occasionally two cells are seen placed together, in the same relative position to each other as the two enclosed in the same capsule, but separated by a septum of ground substance, so that each is enclosed in its own cavity. If, for the indifferent fluid, we substitute distilled water, the cartilage cells separate themselves from the internal surface of the cavity, while their protoplasm becomes turbid. If the cartilage of the newt is subjected to the induction current in the manner already described, a sudden shrinking of the cell results, in consequence of which it assumes a coarsely granular appearance, and a nodulated form, while the nucleus becomes invisible. This condition is permanent, the cell never resuming its former appearance; in some, however, the nucleus becomes more or less invisible. A perfectly similar change is produced by the addition of dilute acetic acid. In many parts of the preparation, especially near the margin, where the cartilage cells are closely packed, the change does not take place. The cells become more transparent, while their edges and those of the nuclei become more sharply defined.

Sections can be easily made of cartilage in the recent state, and can then be examined in an indifferent liquid. The condyles of the tibia or femur of a frog or mammal may be used, or the costal cartilages of the latter. The greatest variety is found in different cartilages, and in different parts of the same cartilage, in respect of the number and size of the cells. For making permanent preparations of cartilage, the chloride of gold method is better than any other. Thin fresh sections of cartilage are placed for ten or fifteen minutes in a half per cent. solution of chloride of gold, exposed to light in distilled water for twenty-four hours or more, and then mounted in glycerin. The matrix remains clear, or is only very slightly stained violet, while the corpuscles display all transitions of colour between violet, violet-red, and dark red. The nuclei are usually brightly stained, of a reddish tint. The method formerly used, which consisted in staining sections of cartilage, previously steeped in chromic acid solution, may be dispensed with; the plan above recommended possessing the great advantage over it, that the cartilage cells retain their natural form completely.—Before leaving hyaline cartilage, a word must be said as to the arrangement of the cells in the cartilages which occupy the centre of the epiphysis of the tibia of the frog. If the tibia of a frog is enucleated from the knee-joint, and sections are made at right angles to the axis of the bone through the condyles, these exhibit concentric layers, arranged around two centres corresponding to the two condyles. Proceeding from without inwards, we have first articular cartilage, then an external periosteum, a ring of bone, an internal periosteum, and, finally, a nucleus of cartilage on either side, one corresponding to each condyle. These two cartilages are hyaline, but each cell constitutes a rigid lamina, which is separated from its neighbours by little or no matrix. Towards the diaphyses each nucleus tapers away gradually; and, in its lower part there is a cavity, which is continuous with the medullary cavity of the diaphysis, and contains a little liquid. The cells are here more separated from each other than they are towards the condyles, but, immediately round the cavity, they are more densely arranged, are roundish in form, and look like lymph corpuscles, consisting of finely granular protoplasm.

In *embryonal cartilage*, the spindle-shaped or stellate branched cartilage cells, which consist of granular protoplasm, and possess spheroidal nuclei, are crowded together in a hyaline substance, penetrated throughout by blood-vessels (*e.g.*, in the patella or head of the femur of the human fœtus). In the immediate neighbourhood of the vessels they possess more or less the form of ordinary cartilage cells. They may be prepared for observation in the same way as the others.

Yellow Cartilage differs from hyaline in the fact that its matrix consists of a network of elastic fibres, in which there are cavities occupied by cartilage cells, either isolated or in groups. These are sometimes surrounded by a certain quantity of hyaline substance. The best objects for the study of this tissue are the epiglottis and the cartilage of the external ear; these may be examined fresh, or in chloride of gold. In addition to these forms, the so-called *parenchymatous cartilage* must be mentioned; *i.e.*, cartilage without matrix. This occurs in the embryonal *chorda dorsalis*, and in the *tendo Achilles* of the frog. We have already studied an example of it in the nucleus cartilage of the epiphyses of the frog's tibia.

Fibro-Cartilage.—In fibro-cartilage the structural elements of cartilage are intermixed with gelatigenous tissue, as in the neighbourhood of the insertions of tendons into bones, in cartilages of the symphyses, &c. The mode of preparation is the same.

Bone.—In the investigation of the structure of bone, one of two courses may be followed, according as we have in view the bony framework, *i.e.*, the bone substance proper, or the soft parts, viz., the periosteum, medulla, blood-vessels, and nerves. The bone substance proper may be studied satisfactorily by means of thin sections, for the preparation of which the method is as follows:—A human long bone, a vertebra, or one of the flat bones of the skull, is cleared of the soft parts and dried. The bone is then fixed in a vice, and thin lamellæ are cut in various directions with the aid of a fine saw. These are rubbed down with moist emery powder on a ground-glass plate, against which they are pressed either with the finger alone, or with a bit of cork, or with a second glass plate, until they are extremely fine. Having been polished on a wet hone, they are washed in water and pencilled with a camel-hair brush, in order to get rid of adhering dirt. They must next be dried and placed under a cover-glass, either without the addition of any liquid, or in glycerin. As examples, transverse and longitudinal sections of a human radius may be taken. In the one, the Haversian canals are seen cut across; in the other, they appear as broad channels, which communicate with each other by cross channels, the latter running obliquely or at right angles to the former.

The clear ground substance consists of lamellæ arranged concentrically around the Haversian canals (primary lamellæ), and secondary lamellæ, which run longitudinally in various planes, occupying the spaces which are left between contiguous systems of concentric lamellæ. The lamellæ contain an immense number of dark cavities (*lacunæ*) at equal distance from each other, which, as longitudinal sections show, are of elliptical form. These communicate with each other by dark, somewhat convoluted canaliculi, many of which run in the same layer, but many also in such a direction as to form communications between one lamella and the next. In dry preparations, the whole system of lacunæ and canaliculi is filled with air. We shall see afterwards that in the living state they contain protoplasmic branching cells.

A second method of preparing bone is that of maceration. A fresh bone is separated from the surrounding muscles and placed in a large quantity of a quarter to half per cent. solution of chromic acid, to which a few drops of hydrochloric acid have been added. The bone acquires a consistence suitable for the preparation of sections with the razor in from a week to a fortnight, according to its size. If too soft, it can be placed in diluted alcohol. Bones prepared in this way may be used just as other tissues hardened in chromic acid (*see* Chap. VI.).

For the study of the periosteum and of the compact bony substance, *i.e.*, of its lamellæ and lacunæ, with the cells contained in them, sections of the long bones of man are very suitable. The spongy substance can be best examined in the metacarpal and metatarsal bones and in the phalanges of children, or in those of rabbits or rats. Very instructive sections may also be obtained from the tibia of the frog, showing the compact substance of the bone, as well as the pigment cells, fat cells, medullary cells, and blood-vessels of the marrow. Medullary tissue can be also advantageously studied in the tongue bone of birds. The whole tongue is hardened in chromic acid solution, after which sections are made through the posterior part of the tongue, so as to pass through the bone in question. Sections of bone prepared as above afford evidence that the cells which occupy the lacunæ are strictly analogous to the branched cells of other connective tissues, so that the system of lacunæ and canaliculi, seen in preparations of dried bone, corresponds entirely with the system of canaliculi (*Saftcanälchen*) seen in silver preparations of the cornea, serous membranes, &c. And it may even be shown in preparations of the flat bones of the skull, or of the tongue bone of birds, that the cells are not only in continuity externally with those of the periosteum (which, although really branched, look spindle-shaped in section), but internally, *i.e.*, towards the medulla, with cells which are also more or less branched, but are arranged so regularly and so close together against the bony surface, that they resemble an endothelial lining. In the flat bones of the skull of human embryos, the same arrangement presents itself with great distinctness—the cells, which line the medullary cavities, being then called *osteoblasts*.

The medullary tissue of bone is rich in blood-vessels and in cellular elements. The former are best seen in injected preparations (*see* Part II., Chapter VI.). After the injected part has been one or two days in alcohol, the bone must be freed from surrounding tissues, and steeped in chromic acid with the addition of hydrochloric acid, as before. The medullary cells, which differ in size and in the distinctness of their granulation, may be examined in the fresh condition on the warm stage, for the demonstration of their amœboid movements, in the manner several times described previously. In chromic acid preparations, the individual medullary cells, as well as the fat cells, retain their form and aspect.

Development of Bone-tissue.—For the study of the development of bony tissue (whether from cartilage or from fibrous tissue, as in the flat bones of the skull) the human fœtus is best adapted, after having been steeped in Müller's liquid, or in one quarter to half per cent. solution of chromic acid, for a few days. The sections may be stained with carmine (*see* Chapter VI.). For studying the development and growth of bone in the epiphyses, longitudinal sections may be made through the epiphysis of the femur, or of the tibia, of the metacarpal bones, or phalanges of newly born human fœtuses, or of young rabbits.

CHAPTER IV.

MUSCULAR TISSUE.

SECTION I.—UNSTRIPED MUSCLE.

THE elements of this tissue are cells—the so-called "contractile fibre-cells"—of varying length, and for the most part spindle-shaped, this form being often modified by a flattening of the cells where they come in contact. Their ends are either single or divided. Their substance is, in the fresh state, a pale or finely granular protoplasm, sometimes longitudinally striated: in the thicker part of the cell lies an oblong, compressed nucleus, rather rounded at the extremities (thus becoming staff-shaped), or pointed. The nucleus contains one or two large shining nucleoli: if single, the nucleolus lies in the centre of the nucleus; if double, one is found at each extremity. External to the nucleus, and in a straight line with its longitudinal axis, some small granules may sometimes be seen. The unstriped muscular fibres are always arranged in bundles, the elements of which are separated from each other by interstitial substance. The bundles are held together by connective tissue, in which they lie in such a way that they either form membranes (as in the intestine) or meshworks (as in the bladder). In the former case, the bundles are parallel and mostly undivided; in the latter, they run in various directions, divide frequently, and intercommunicate with each other.

The best materials for the study of involuntary muscular fibre, are the bladder of the frog, the mesentery of the newt, the muscular coats of the intestines of the frog and mammalia, and arteries, such as those at the root of the mesentery of the frog. They may be demonstrated either in connection or isolated.

To show their arrangement, a portion of the bladder of the frog may be spread on the glass slide with the mucous surface downwards, and covered in half per cent. salt solution. In such a preparation it is seen that a meshwork is formed by the repeated division of the bundles of fibres. If the bit is soaked for a few minutes in one per cent. or two per cent. acetic acid, the epithelium brushed off with a camel-hair pencil, and the membrane then examined in water or glycerin, the individual elements of the muscular bundles come very distinctly into view; those of the muscular coats of the arteries can also be studied advantageously. The mesentery of the newt may be prepared in the same manner. Instructive preparations of muscular tissue may be obtained by carefully excising the iris of an albino rabbit just killed, and spreading it flat on the object glass in an indifferent liquid. The muscular tissue of the intestine can be prepared as follows:—A short portion of the small intestine of a rabbit, or mature fœtus, is filled with half per cent. salt solution by ligaturing one of the ends, and tying into the other a glass tube with a cannulated extremity, through which the liquid must be injected. The gut having been in this way well distended, a second ligature is placed between it and the cannula. Thin shreds, consisting only of the peritoneum and of the longitudinal muscular layer, are then stripped off with the aid of pointed forceps from the surface of the intestine opposite to its mesenteric attachment. These strips are carefully spread out and prepared in an indifferent liquid. Of course care must be taken not to pierce the intestine with the forceps. Another suitable object of study is the abdominal extremity of the Fallopian tube, which, in some mammalia (*e.g.*, in the sow), is dilated into a large thin sac. It may be prepared in the same way as the bladder of the frog. An excellent method of preparing unstriped muscle is to immerse the tissue in half per cent. solution of gold, for which purpose the bladder of the frog, the mesentery of the newt, the iris of the eyes of albino animals, or the muscular coat of the intestine of small mammalia may be used. The bladder of the frog is prepared as follows:—A frog is decapitated, and the upper two-thirds of the abdominal cavity opened. A solution of chloride of gold is injected, either by means of a tube ten to fifteen centimeters in length, which is drawn out at one end so as to form a cannula, and bent at an obtuse angle so as to facilitate its introduction into the bladder, or, still better, with the aid of a glass syringe furnished with a long beak. As soon as the bladder is filled, a ligature is placed round its neck and tightened round the cannula, after which the organ may be excised and placed in a capsule, containing a similar solution, for fifteen minutes. After this time it is cut into small sections, which are immersed in acidulated water and exposed to the light. If this method is followed, there is no fear of folds or shrinking, as the bladder is already more or less hardened. As soon as the fragments have acquired a dark violet or dull red colour, the mucous membrane is pencilled away and the remainder covered in glycerin. The mesentery of the newt, the iris, or the muscular coat of the intestine of mammalia may be prepared in the method already explained, and then treated in every respect as just described. Chloride of palladium, which has also been used for the colouring of muscular fibres, has no advantage over the gold salt. In sections of unstriped muscle, previously hardened in one-eighth to one-fourth per cent. solution of chromic acid, and subsequently coloured in picric acid, carmine, aniline, &c., the muscular bundles are distinctly seen, as well as their relations to each other, and to the septa of connective tissue which surround and separate them.*

In sections through the hardened intestine of the frog, rabbit, or rat, the muscular cells, where they are seen in longitudinal section, appear to be separated from each other, not by straight lines, but by marginal borders, which exhibit fine transverse markings, referable to the existence of minute furrows, which run in a direction vertical to the axis of the fibre.

To isolate the individual muscle-cells for the study of their form and nuclei, macerating liquids, by which the interstitial substance is disintegrated, are employed. Small fragments are introduced into a dark sherry-coloured solution of bichromate of potash, two or three per cent. acetic acid mixture, nitric acid diluted with four times its volume of water, or thirty-five per cent. potash solution. The arrangement of the nerves of unstriped muscle will be described in a future chapter.

SECTION II.—STRIPED MUSCLE.

The tissue of striped muscles consists of long cylinders (muscular fibres) which are united by connective tissue into bundles (fasciculi) of varying length. The following parts have to be considered :—The contents or substance of the individual fibre with its muscle-corpuscles; the sarcolemma; and the junction of muscle with tendon. The mode of ending of the nerves in muscle will be described in the next chapter.

Proper Substance of Muscular Fibre.—The leg of a water-beetle (*Hydrophilus*) is torn out, and its horny covering removed. A snip is then taken from the exposed muscular mass, with the aid of curved scissors, or a fine scalpel, and at once covered without addition. If the cover-glass is then slightly pressed, so as to flatten out the object, arborescent branchings of the tracheæ first attract attention. These air-tubes consist, like the tracheæ of mammalia, of parallel rings, and entwine the muscular fibres with a network of fine, dark capillaries, each of which follows a winding or spiral course. The muscular fibres themselves, which either run parallel, or cross each other in various directions, are in active movement. In some fibres this movement resembles that of a wave, which rapidly progresses in the direction of its length; in others, when it is slower, it has a vermicular character. On more careful examination it is seen that, during the progress of the wave, the muscle swells, returning to its original thickness immediately after. It is further observed that the dark parallel striæ come nearer together during the swelling, and that the intervals return to their original width after the wave has passed. In the contents of a muscular fibre, when in a state of rest, the following parts can be distinguished: (*a*) the dark, parallel cross stripes, which, as we shall find, correspond to thin parallel disks of less refractive isotropous substance (called interstitial disks); (*b*) the portion intervening between these. This, again, appears to consist of two parts, viz., a broader middle one of dull grey appearance, and on either side of this a narrow, clear layer. The whole is made up of highly refractive, anisotropous, contractile substance, which is to be regarded as the essential substance of the muscular fibre. The dark cross-lines do not seem, under high powers, homogeneous, but appear to consist of series of contiguous granules of equal size. Many muscular fibres exhibit no other differences; in others, it is possible to distinguish lines running longitudinally, of greater or less extent, and which are so arranged that they come between what appear to be dark granules of the interstitial striæ. With reference to these granules, it is not to be supposed that they actually exist as such; the appearance is rather to be regarded as expressive of the fact that the dark, interstitial transverse stripes are interrupted by clear, longitudinal lines, the interval between the latter remaining dark—as, *e.g.*, in a check of which dark transverse lines are covered by light longitudinal lines. In a fresh muscular fibre, as seen under the microscope, the transverse interstitial disks are not placed vertically, as we can satisfy ourselves by using the fine adjustment, but are set at an oblique angle with the long axis of the muscular fibre. In this respect a muscular fibre may be compared to a roll formed of coins of different metals, so arranged that the thin dark disks alternate with thicker light ones. If such a roll is laid on a plain surface, all the coins lean in one direction, and present their edges to the eye, regarding them from above, just as the disks in a muscular fibre do under the microscope.

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* Fine sections of structures containing numerous unstriped muscular fibres, which have been hardened in chromic acid, then placed for a few days in diluted alcohol, and finally stained in a weak ammoniacal solution of carmine, exhibit a striking contrast between the muscular fibres and the connective tissue, the former being tinged yellow by the chromic acid, the latter red by the carmine.

We have now to consider the significance of the appearances above described. The fact may be stated *in limine*, that the whole of what intervenes between two interstitial stripes, *i.e.*, the grey band and its two bright borders, affects polarised light in the same way—that consequently the view according to which only the borders are doubly refracting, is erroneous. If a microscope is employed, of which the stage admits of rotation around the vertical axis of the instrument, as in the larger instruments of Hartnack, and these bright borders (which should be distinct and regular) are observed, in a muscular fibre, under No. 8 objective, and if the stage is slowly rotated, so as to alter the course of the rays in relation to the muscular fibre under observation, a remarkable change is seen to take place in these borders. As the rotation is continued the bright bands fade, first on one side of the interstitial line, then on the other, coming into view again in the same order; the changes do not, however, occur simultaneously throughout the whole of the muscular fibre.

When fresh muscular tissue is placed in absolute alcohol, and then steeped for a few minutes in oil of turpentine, mounted in dammar varnish, and covered, only two kinds of substance can be distinguished in the fibres, *i.e.*, dark interstitial stripes and a dull grey substance between them, without a trace of the clear borders. The longitudinal section of such a muscular fibre may be represented diagrammatically, as in Fig. 15. In the diagram, the slightly refracting interstitial substance is represented by *a*, the clear borders by *b*, the dull grey by *c*. Let us endeavour to understand the course of the rays which pass through *b*. Let ς be a ray which enters from the mirror in the direction $\alpha\ \beta$, and penetrates at o into the less refractive medium *a*, and passes through it in the direction $o\ \delta$—inasmuch as it deviates from the normal in *a*. Let ς' be another ray which enters *b* at the angle m', *i.e.*, a greater angle than that under which ς enters. Accordingly, the deviation it will undergo in the medium *a* will be greater than the deviation undergone by ς. And if it be assumed that it is so great that the sine of the angle of deviation $= l$, the angle being a right angle, the ray will pass out between *a* and *b*. If the angle of incidence is greater than m' the angle of deviation is greater than a right angle, so that the ray does not enter *a* at all, but is totally reflected through *b*. Hence the substance *b* appears clearer than *c*, for more rays pass out at *b* than at *c*; the excess consisting of those rays which, entering *b* in a direction towards *a*, and at a greater angle than m', are totally reflected in *b*, as above explained.

The bright borders of the proper substance present the same characters and relations in the muscles of crabs after treatment with gold. Occasionally they may be also seen in the muscles of frogs, and in those of the tail of the rabbit, if quite fresh. From these facts it is evident that the clear borders of the proper substance need not be regarded as actually anatomically distinct from the rest, but their presence can be explained as mere optical results of total reflection, *i.e.*, provided it be admitted that the interstitial substance and the proper substance refract light in different degrees.

In order to study the longitudinal striæ, preparations must be made in humor aqueus of the fresh muscular tissue of *Hydrophilus*, of the sartorius of the frog, or of the muscles of the back of the lizard, care being taken to separate the muscular bundles slightly from one another. In such preparations it is seen that the substance which lies between two adjoining transverse striæ appears to be marked off into a number of quadrangular areas, which correspond to the sides of the prismatic "sarcous elements."

A number of such sarcous elements, arranged in a linear series parallel to the axis of the muscle, and connected each to each by shorter disks of transparent intermediary substance, together constitute a so-called primitive fibril. And, in accordance with this definition, we can conceive each muscular fibre to be formed of primitive fibrils, along with the intermediary substance (corresponding to the longitudinal striæ), by which these fibrils are held together. It is no less possible to conceive of the muscular fibre as consisting of disks (each composed of a number of laterally contiguous sarcous elements, along with the intermediary substance by which they are, as just remarked, held together), separated each from each by thinner disks of intermediary substance. The best demonstration that the sarcous elements are the elements of the muscular substance, which are arranged in disks transversely, and in fibrils longitudinally, is to be obtained by the method of Cohnheim. A muscular fibre of a frog, *Hydrophilus* or cray fish, is exposed in a platinum capsule to a freezing mixture, at a temperature of 6° C. to 8° C. After a short time the muscle acquires the consistence of wax. Fine sections are then made with the aid of a cooled razor, and are at once examined in a drop of serum under a thin cover-glass, care being taken to introduce slips of silver paper to avoid pressure. Such a preparation, seen under Hartnack's immersion objective No. 10, exhibits the following facts:—Circular or oval disks present themselves (cross sections of muscular fibres), the margins of which are sharply defined, and possess a double contour (sarcolemma). Within the sarcolemma a beautiful mosaic is seen, in which the triangular, four-sided, or pentagonal areas appear to consist of dull-looking material, separated by lines which are brighter, more transparent, and refract light less strongly. These lines are in general of extreme tenuity, but certain spots are always to be observed, within which the areas of dulness are further apart; in other words, the clear lines of demarcation are wider. Wherever this is the case, there exist sharply defined nucleus-like bodies, which, as we shall find, are actually the nuclei of the muscle-corpuscles. In cross sections of muscular fibres of crustacea, insecta, amphibia, and reptilia, nuclei, surrounded by spots in which the clear lines are thicker than elsewhere, are met with in all parts of the fibres; but in mammalia they occur only in the immediate neighbourhood of the sarcolemma. In the crustacea and in *Hydrophilus*, the prevalent form of the mosaic is pentagonal; in the frog, four-cornered, and usually rectangular. Provided that the preparation is protected from pressure and evaporation, it remains unaltered for several days. If a small quantity of water or very dilute acetic acid is added to the fresh preparation, the disks swell out in a remarkable manner; the polygonal areas become more transparent and increase in size, while the intermediary substance disappears.

A fresh section, obtained as above, may be placed for a few minutes in diluted serum, and then transferred for from ten to thirty seconds to half per cent. silver solution; finally, washed in water slightly acidulated with acetic acid, covered in glycerin, and exposed to light. A preparation is thus obtained in which the sectional disks are coloured of various shades, from clear yellowish-brown to dark brown. Clear white lines on a brown ground are seen with great distinctness, which correspond completely with the trellis-work of transparent lines seen in the fresh preparation, from which appearance we learn that the spaces of the mosaic are stained brown by silver. Oblique sections, whether examined fresh or after staining with silver, exhibit corresponding appearances. In longitudinal sections, prepared according to the same method, small brown rectangles, longer in the direction of the axis of the muscle than in the transverse direction, which correspond to the sarcous elements, are here and there visible. These rectangles are separated from each other by clear narrow lines.

If a very small fragment of mammalian or frog muscle (sartorius or mylohyoid of the frog, or the flat muscle in front of the trachea of the rabbit), be steeped for fifteen or twenty minutes in chloride of gold, then exposed to light for one or two days in slightly acidulated water, and subsequently hardened in common alcohol, sections can be made in planes at right angles to the axis of the muscle. These exhibit appearances which coincide in every respect with those above described, the only difference being that the rectangular sarcous elements exhibit a clear red or purple tinge, while the interstitial substance is dark.

From all these facts we learn that the substance of a muscular fibre consists, in the first place, of oblong prisms, *i.e.*, sarcous elements, with their axes parallel to its axis, and formed of a material which refracts light strongly, is stained strongly with silver, slightly with solution of chloride of gold, and swells out in the fresh state on the addition of water; and, secondly, of a less refractive, transparent, interstitial substance, occupying the remainder of the space; which is not coloured by silver, but is intensely stained by chloride of gold, and disappears in dilute acetic acid. This last reagent appears to have the faculty of dissolving the interfibrillar part of the interstitial substance, leaving the interstitial disks of the fibrils almost intact. Similar facts are observed in muscles which are subjected to the hardening influence of alcohol or chromic acid. In sections of muscles so prepared, the fasciculi which are cut transversely, are seen to consist of disks, which are either round, or flattened against each other, and may be easily stained in carmine or picric acid. In such disks the double contoured section of the sarcolemma includes a number of small roundish corpuscles, each of which, as may be seen in longitudinal sections, is a fibril cut across. Muscular fibres, cut longitudinally, seem to consist merely of fibrils which are divided by cross lines into small long rods placed end to end. In sections of hardened tongue of the frog, it is very easy to obtain isolated fibrils: they are also to be seen in teased preparations of other muscles hardened in alcohol and chromic acid.

The Sarcolemma.—Each muscular fibre is invested in a structureless hyaline membrane. To demonstrate it, the readiest method is to add water to a fresh preparation of *Hydrophilus*, or better, frog-muscle. After a short time the sarcolemma separates in transparent bulgings with double contours. Greater lengths of sarcolemma can be shown, by carefully teasing fresh frog-muscle in salt solution. In such a preparation, fibres are always to be found, which, over a greater or less extent, are no longer striated, but consist of a finely granular mass. Continuing the observation, it is seen that the parts of the fibre on either side of such a spot become contracted, as indicated by the approximation of the transverse striæ, and by the widening of the fibre. By virtue of this contraction, the granular muscular substance is torn asunder, the sarcolemma being brought into view as a transparent tube. Within this tube a greater or less number of granules are observed in active molecular movement. As the disintegration of the muscular substance progresses, an increasing quantity of sarcolemma is brought into view. The broken up ends of muscular substance are always irregular in form, presenting numerous projections, none of which exhibit striation. By and by fresh spots become the seat of the same change, so that the disintegrated parts are separated from each other only by short intervals of normal muscle. By drawing asunder a small number of muscular bundles, their opposite ends being seized with fine forceps, a preparation may be obtained which shows similar appearances in a larger proportion of fibres.

The extraordinary power of resistance of the sarcolemma may be shown as follows:—One of the hind legs of a tadpole is amputated at the thigh. The animal is then replaced in water. After forty-eight hours, the loosened muscular fibres hang from the stump in long pencils. If these are cut off close to the surface of the stump with sharp scissors, and covered in water, they are found to consist of a number of hyaline tubes, which, when seen in profile, present doubly contoured edges. Next the cut edge some of them contain a plug of striped muscular substance, or of coarsely granular material, which is divided into a number of closely packed polyhedral cells. In the rest of the tubes, coarsely granular young cells are seen sprouting from the internal surface.

Muscle-Corpuscles.—In preparations of fresh muscle (newt, frog or *Hydrophilus*) numerous nuclei occur, which in the *Hydrophilus* are roundish, in the frog oblong or staff-shaped. If dilute acetic acid be added, the muscular substance becomes swollen and transparent, and the nuclei are seen very distinctly, each embedded in granular protoplasm, which has the form of a spindle-shaped cell, the long axis of which is parallel to that of the fibre. If, on the other hand, we examine an oblique or cross section of frozen muscle, covered in dilute acetic acid, it is easy to satisfy one's self that the nuclei in question are not embedded in fusiform protoplasmic masses, but in finely granular lamellæ, which are seen to be dotted about the whole thickness of the fibre, and may be either divided or simple. The distribution of these lamellæ in the muscular fibre differs in different animals. In mammalia, they are confined to the immediate neighbourhood of the surface; in the *Hydrophilus*, crab, newt and frog, they constitute a network within the muscular fibre, exhibiting marked differences in thickness, not only between different lamellæ, but between different parts of the same lamella. In fresh muscle of *Dytiscus marginalis*, the arrangement of these protoplasmic masses is as follows:—In some muscular fibres, the granular protoplasm has, throughout the fibre, the form, more or less, of cylindrical bands, in which roundish nuclei are arranged close together in linear series. Here and there, these nuclei are separated by distinct marks, so that the whole cylinder seems as if divided into portions, each corresponding to a nucleus. In other fibres, there are, in place of an axial cylinder of protoplasm, two or three lamellæ, which are continuous with each other by subordinate lamellæ of various extent. In these, roundish nuclei are embedded at various distances, and in cross sections they appear thicker at the level of the nuclei. In an optical longitudinal section, in which a lamina is seen in its whole length, it is observed to be usually curved. In a transverse section it is also often curved. We therefore conclude that these lamellæ are composed of placoid cells, each of which corresponds to a nucleus, and constitutes a *muscle-corpuscle*, the limits of which are indicated by the markings often seen between neighbouring nuclei. In *Hydrophilus*, muscular fibres are also met with, in which the lamellæ are replaced by cylinders.

In the individual muscular fibres of the tongue of the frog, obtained by taking a snip from that organ near the surface, and covering it at once with serum, chains of oblong nuclei, or large groups of nuclei without definite arrangement, are to be found here and there. In the latter case, the nuclei are not all oblong; some of them are constricted and possess knobs. In sections of tongue stained in gold, it is seen that these chains and groups of nuclei are embedded in granular protoplasm, which is continuous with the granular lamellæ above described. These bodies are therefore to be regarded as enlarged, many-nucleated muscle-corpuscles.

Tendinous Insertions.—The transition from muscle to tendon takes place in two ways:—In one the transverse striæ cease, the whole muscular fibre passing into a tendinous bundle of the same size, consisting of parallel wavy fibres. In the other, the muscular fibre tapers to a blunt point, the sarcolemma extending beyond it as a thread-like structure of varying thickness, resembling, and becoming continuous with, a slender bundle of connective tissue. Oblong cellular structures may be seen in this fibre. The first form may be very easily and completely demonstrated in a teased preparation in serum or saline solution, in the muscular layer which extends, in *Hydrophilus*, from the trunk to the first joint of the extremities, or in a similar preparation of the thoracic cutaneous muscle of the frog. In the latter case, care will be necessary to remove the tendinous insertions along with the muscle, and to spread out the whole in serum or saline solution before covering it. The second form can be studied in fresh teased preparations of the muscles of the limbs of small mammalia, or of the muscles of the larynx; but more easily in very thin sections of the tongue of man or of mammalia, especially in those fibres which radiate upwards towards the dorsal mucous membrane. In sections of tongue hardened in chromic acid, which are made across the long axis of the organ, bundles of fibres are seen to pass upwards between the transversely cut bundles of the *longitudinalis linguæ*. Of these bundles it is seen that certain of the muscular fibres stop short, the sarcolemma being prolonged into a thread, as above described. The rest of the muscular fibres enter the *mucosa*, and end in tendinous bundles of equal diameter, which again unite with the meshwork of the *mucosa*.

Arrangement and Division of Muscular Fibres.—They are grouped into bundles by septa of connective tissue, which in general contain numerous amœboid cells, and a network of ordinary branched cells. From these septa thinner lamellæ spring, which are interposed between the individual bundles. In a mature fœtus a cross section of muscular bundles, *e.g.*, of the tongue, palate, or eyelids, shows that they are intersected by a beautiful network of nucleated branched cells, in such a way that each mesh is occupied by a single fibre. In general, striated muscles do not divide: there are, however, situations in which muscular fibres are seen to divide dichotomously or dendritically. The best example is to be found in the cardiac muscular fibres, of which a repeated dichotomous division is characteristic, as also their union with one another so as to form a network. In the tongue of mammalia, the muscular fibres often divide before ending in tendons; but in that of the frog the divisions occur much more frequently. Both in recent preparations, and in sections made after hardening, muscular fibres are seen which branch dendritically, as they ascend towards the dorsal mucous membrane, the ultimate branches being so small that they contain only a few fibrils, which finally end in connective tissue fibres.

Examination of Muscular Fibre in Polarized Light.—We assume the reader to be acquainted with the action of a Nicol's prism, contenting ourselves with stating that the polarization microscope is an ordinary microscope, in which one Nicol is placed above the eye-piece or ocular (*i.e.*, between the eyeglass and the observer's eye), and a second between the object and mirror. The upper Nicol is usually of one piece with the ocular. The prism is so fixed that it can be rotated, and that the axis of rotation is contained in its principal plane. The degree of rotation is measured by a graduated circle. The lower Nicol is surrounded by a condensing lens, and can (in Hartnack's microscope) be fitted into the tube which ordinarily contains the diaphragm or condensor. In looking through such a microscope, it is seen that the illumination of the field varies according to the relative position of the two prisms; so that, in rotating the upper one (which is called the analyzer), it is darkened and lightened twice in each complete rotation. The positions of greatest obscurity are those in which the principal planes of the two Nicols are at right angles to each other—of greatest luminousness, those in which these planes are coincident. When the microscope is used with the Nicol in the first-mentioned position, the object is said to be observed between crossed Nicols.

Before proceeding to describe what is seen in muscle when examined between crossed Nicols, the facts observed when crystals which possess similar optical properties are looked at in the polarizing microscope, should be first carefully studied. Muscular fibres can be shown to possess optical properties which resemble those of doubly refractive, positive, uniaxial crystals, such, *e.g.*, as those of rock crystal or quartz, &c. The meaning of these expressions must be illustrated. If a number of doubly refracting microscopical crystals of any kind are placed under the polarizing microscope, it is seen that when the upper Nicol (or analyzer) is rotated so as to make the field dark, the crystals appear (according to their position) more or less illuminated; whereas this is not the case if the crystals are isotropous, *i.e.*, belong to the "regular" system of crystallization.

The degree of illumination of each crystal varies according to its position. This may be readily shown by rotating the object-glass, or stage on which it lies, without moving either prism: it is then seen, as regards each crystal (supposing the Nicols to be crossed), that four times in each complete rotation it loses its luminousness altogether. These two positions are called the inactive azimuths, because the body looks in these positions as if it were isotropous—dark on the dark field. This happens whenever the principal plane of the crystal lies in the principal plane of either Nicol, and is consequently at right angles to that of the other. In all other positions it looks more or less illuminated, the degree of brightness increasing and diminishing as the azimuth in which it is placed declines or approaches; consequently, the crystal appears brightest when its principal plane is inclined at an angle of 45° to the plane of polarization. When the crystalline body is of a certain thickness, the appearances are somewhat different. Thus, if a plate of mica from one to two millimeters thick is placed on the object-glass with the Nicols crossed, it is seen that the field is not only luminous but coloured, the colour varying according to the thickness of the plate—its intensity varying according to the inclination of the principal plane of the mica to that of the prisms, being brightest when that inclination is 45°. If now the plate of mica is rotated, it is seen that in each rotation, as before, there are four azimuths of greatest brightness, and four intervening ones of greatest obscurity. But, in addition to this, it is observed that, in the bright azimuths, the colours displayed differ—the colour of the field in any given position of the plate being complementary to that seen when it is rotated 90°.—These facts are of great practical importance in all cases in which it is desired to observe the doubly refractive parts of transparent objects between crossed Nicols, without losing sight of those parts which are isotropous. If such objects are examined in the ordinary way in the dark field, it is obvious that all those parts which are isotropous are invisible. If, however, the field is coloured, by placing a plate of mica or selenite underneath the object, everything is seen as distinctly as if the light were not polarized, with the difference that the doubly refractive bodies are distinguished from others by their colour—the latter being of the colour of the field, the former of a colour differing from it variously, according to their thickness, their position, and their optical properties. In all doubly refracting crystals, there is at least one direction in which light may be transmitted without suffering double refraction—*i.e.*, bifurcation. Those crystals in which there is only one such direction are called uniaxial, *e.g.*, Iceland spar, quartz, and tourmaline. When such crystals are examined between crossed Nicols, in such a position that the light is transmitted through them in the direction above referred to (which is always that of the axis of crystallization), they are not seen. We shall find that the same holds good as regards the anisotropous parts of muscular fibre.

In a fresh muscular fibre seen between crossed Nicols, the first fact that strikes one is that the appearances correspond with those observed in doubly refracting bodies. Next it is seen that all the muscular fibres under observation are not equally illuminated. Those are brightest which are so placed that the long axis forms an angle of 45°, the illumination diminishing as the angle diminishes, until it disappears at the moment that the fibre axis lies in the plane of polarization of either Nicol. It is further seen that all parts of the muscular fibre are not doubly refracting, but only those parts which were before described as sarcous elements. The interstitial substance looks dark whatever be the position of the fibre, so that between crossed Nicols it is invisible.

The method to be adopted for demonstrating these facts is as follows :—

Method.—From a number of plates of selenite or mica, one is selected which, when placed in the proper azimuth, gives between crossed Nicols a field of the tint which is known as *teinte de passage*.* Such a plate having been found, it is fixed to the object-glass with a drop of dammar. Fresh muscular fibres of the extremities of the crab, frog, or *hydrophilus*, are placed in absolute alcohol for half-an-hour, or in ordinary alcohol for several days. As soon as the muscular tissues are deprived of water, they are soaked in oil of turpentine. Of the muscle so treated, a preparation is made on the plate of mica above mentioned, the muscular fibres being teased in such a way that they lie in various directions. If the preparation is now examined in the purple field (obtained as above described), the different colours of the individual muscular fibres are brought out with the greatest distinctness. On rotation of the upper Nicol they undergo changes; if the rotation extends to 90°, each colour is replaced by its complementary. In a cross section of a muscle (of any animal) hardened in alcohol, and prepared in dammar varnish after steeping in turpentine, the individual fibres show various degrees of illumination. All transitions present themselves between those which are bright between crossed Nicols, and those which are completely invisible; and it is found that the latter are those which are cut in planes at right angles to their axis—the former, those cut at an angle of 45° to their axis. It is thus seen that the long axis of a muscular fibre corresponds, in relation to its properties of double refraction, to the axis of crystallization of a uniaxial crystal—in short, that a muscular fibre is optically comparable to such a crystal. Brücke has further demonstrated, not only that the muscular fibres are uniaxial, but also that they are positive, *i.e.*, that they resemble those uniaxial crystalline bodies in which the extraordinary index of refraction exceeds the ordinary index. Inasmuch as the apparatus necessary for demonstrating this is not to be found in most laboratories, and an explanation of the mode in which it is accomplished would involve a more general discussion of the subject of polarization than our space allows, it has been omitted. The reader is referred to Brücke's article in Stricker's Histology for further information.

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* The *teinte de passage* is a peculiar purple violet, and lies between red and blue in this sense, that if the plate possess a thickness a shade greater than that which

If a teased preparation of the fresh muscle of a frog is treated with water and covered, the ends of the muscles swell, and the contents project as a transparent granular mass, in which the striæ are no longer visible. Between crossed Nicols these parts are found to be doubly refractive, and look like a silver-grey cloud of dust on the dark ground. The particles of which the cloud consists are regarded by Brücke as the real elements of the doubly refracting substance. They are the constituents of the sarcous elements, and are called Disdiaklasts. The disaggregation of the sarcous elements is determined by the water.

produces the tint required, the colour is blue; if a shade less, red. These facts are of importance as aids in selecting a plate.

CHAPTER V.

TISSUES OF THE NERVOUS SYSTEM.

SECTION I.—NERVE FIBRES.

According to the presence or absence of the medulla which surrounds their central and essential part, viz., the axis-cylinder, nerve fibres are distinguished as medullated and non-medullated. The presence or absence of the so-called Schwann's sheath affords an additional and subordinate distinction. This sheath is a resistant, elastic, sometimes fibrillated, but more commonly homogeneous, membrane, containing a variable number of oval nuclei.

Axis-Cylinder.—All nerve fibres contain an axis-cylinder: it is a solid cylindrical structure, which, under the highest powers, is seen to be made up of the most delicate fibrils (primitive fibrils). It varies in size, in accordance with the thickness of the nerve fibre. As it approaches the periphery, it splits into its constituent fibrils by repeated division, or by giving off smaller lateral branchlets. To demonstrate the fibrillated structure of the axis-cylinder, a fresh nervous bundle may be prepared from the lateral columns of the spinal cord of a small mammal, from the optic nerve, the olfactory nerve, or from some nerve belonging to the sympathetic system. The preparation must be macerated for twenty-four hours in iodized serum, and then further prepared by teasing with needles. In the nerve fibres of the lateral columns of the spinal cord, the structure of the axis-cylinder may also be shown in preparations which have been steeped for several days in diluted solution of bichromate of potash. In preparations thus obtained, many of the fibres are seen to exhibit points at which the medullary sheath is broken, in consequence of which the pale, finely striated axis-cylinder becomes visible. Fibrillar structure may also be readily demonstrated in the processes of the ganglion cells, and in the pale naked axis cylinders of various thicknesses of the nervous centres. Again, in the fresh tadpole's tail, as prepared in serum or in half per cent. salt solution, fibrillar structures can be seen with great distinctness in the peripheral branching axis-cylinder. This structure is not, however, peculiar to the peripheral or central portions of the course of a nerve, but exists in other parts. To show this, the best way is to place the fresh nerve in common alcohol for a few minutes, and to stain the preparation with carmine. It must then be put in absolute alcohol for twenty to thirty minutes, after previously teasing it out somewhat. If it is allowed to remain twelve hours or more in oil of turpentine, and then covered in Dammar varnish, it will be found that all the nerve fibres are more or less completely deprived of their medullary sheaths. The axis-cylinder appears in general to consist of granulous substance, but here and there distinct longitudinal streaking can be recognized. The axis-cylinder can also be freed of its medullary sheath if chloroform or collodion be added to a teased preparation of fresh nerve, which is as nearly dry as possible without being thoroughly desiccated. Occasionally the primitive fibrils of the non-medullated nerve fibres are beset with small varicosities at nearly regular intervals, which, when treated with certain reagents (perosmic acid, chloride of gold), become very distinct.

Medullary Sheath.—In a teased preparation of a fresh sciatic nerve of the frog, in half per cent. salt solution, the individual nerve fibres are seen to be invested by a sheath of transparent, highly refractive material, which, when it presents its surface, appears hyaline, but, as seen at the edge of the nerve, exhibits a double outline. Thus the medullary sheath confers on the nerve fibre a dark edge or double contour; so that these appearances in a nerve are characteristic of its presence. Soon after the preparation has been made, it is observed that the sheaths of many fibres become beset with drop-like bodies of irregular form, which are either bright and shining, or granulous and turbid. They are produced by a coagulation of the medulla. In preparations made in iodized serum, the fibres remain, however, for several hours quite smooth, without undergoing this change. In the nervous centres, the medullated fibres which possess no Schwann's sheath often present a necklace-like appearance, due to this coagulation of the medullary sheath (the so-called varicose fibres). The medullary sheath exhibits a remarkable arrangement at those points of the course of the nerve at which it divides into two or more branches. At such points the sheath becomes considerably attenuated, as well as contracted. To show this, the membrana nictitans of a frog is carefully excised, spread out in a drop of humor aqueus, and covered—care being taken to introduce strips of paper under the cover-glass so as to prevent pressure. The thoracic cutaneous muscle of the frog may be prepared in the same way.

Where a medullated nerve fibre passes into a non-medullated, as in the objects above mentioned, the sheath is usually thinned out towards the point where it is about to cease, in which case the thin portion may either extend up to the line at which it abruptly terminates, or may end in a terminal thickened border. In other instances, more particularly in the striated muscles, the sheath very often stops suddenly without any previous attenuation.

Neurilemma.—In order to make out satisfactorily the relation of the nerve fibres in a nerve trunk, sections must be prepared, either of nerves hardened in alcohol, or in diluted chromic acid, and must then be stained with carmine; or tissues known to be richly supplied with nerves must be employed, *e.g.*, tongue, œsophagus, trachea, urinary bladder, &c. In cross sections of nerves, the nerve fibres are seen to be enclosed in a well-defined connective-tissue sheath (neurilemma), of thickness more or less proportional to that of the nerve itself. Between the fibres of the neurilemma, cellular structures are met with. In many nerve trunks, septa stretch inwards from the sheath, by which the nerve fibres are divided into a greater or less number of bundles. In such preparations the cross sections of each nerve fibre exhibit an external ring with double contour—the cut edge of the medullary sheath—enclosing a body of circular outline which does not fill up the whole of the space, and is readily stained by carmine. In a longitudinal section of a nerve we observe, within the connective-tissue sheath, the double contoured fibres, running parallel with each other, but following a more or less wavy course, and showing the nuclei of their Schwann's sheaths. In newly-born children the number of nuclei is much greater than in adults. The spinal nerves, which in the frog find their way to the skin from the spinal cord through the dorsal lymph sac, possess an extraordinarily thick neurilemma; this is covered by a layer of endothelium, which can be demonstrated by staining with nitrate of silver.—In the neurilemma of many microscopical nerves, fine capillary vessels can often be made out. For the tracing out of medullated fibres, the use of osmic acid is of great value; for the medullary sheath is, in consequence of the fatty matter it contains, stained dark by this reagent.

Schwann's Sheath.—With the exception of the optic and auditory nerves, the fibres of all peripheral nerves possess a Schwann's sheath. The nuclei which the sheath contains are seen, when examined in the fresh state in indifferent fluids, to be pale, and more or less distinctly granular. When acted on by acids or hardening reagents, they shrink. In freshly prepared teased preparations (*e.g.*, of the sciatic nerve of the frog), the

Schwann's sheath of the wide medullated fibres can be recognized with great difficulty. In general, only the nuclei can be made out. The sheath itself can be more easily seen in the narrow non-medullated fibres. In the nerves of the tail of the tadpole, and of the membrana nictitans of the frog, in those of the mesentery of the frog and of mammalia whether in the fresh state or treated with gold, in the cornea of the frog or of mammalia treated with gold, in sections of the epiglottis or of the mucous membrane of the mouth made after treating the tissue with gold—the Schwann's sheath can be often recognized as a more or less distinctly streaked membrane. It generally ceases where the non-medullated fibres split into their constituent fibrils.

Non-medullated Fibres.—Various methods must be used for the demonstration of the non-medullated fibres, for the same method does not answer equally well in all cases. Among these, chloride of gold has, unquestionably, the first place. In very many instances it affords the only means we have of following these fibres to their finest ramifications, *e.g.*, in the skin, mucosa, cornea, and striped muscular tissue, &c. Osmic acid is also very useful. The silver method, or treatment with certain acetic acid mixtures, is occasionally employed. In membranes which are prepared in the fresh state in an indifferent liquid, individual non-medullated nerve fibres can be seen, but their finer ramifications cannot be traced, even in the most transparent, without the aid of the reagents above mentioned.

SECTION II.—NERVE CELLS.

Nerve Cells, *i.e.*, ganglion cells, may be investigated (*a*) in the ganglia which are attached to the spinal and certain cerebral nerves; (*b*) in the grey substance of the brain and spinal cord; (*c*) in ganglia belonging to the sympathetic system.

(*a*) **Ganglia of the Cranial and Spinal Nerves.**—As may be seen in sections of these ganglia (hardened in chromic acid or Müller's fluid), each of them is enclosed in a capsule. This capsule varies in thickness in different ganglia, and is continuous with the neurilemma of the nerves which enter and leave the ganglion. It consists of fibrillated connective tissue, in which the cellular elements proper to that tissue may be distinguished. From it septa of connective tissue stretch inwards, and unite by anastomosis so as to form a meshwork. This meshwork serves to support the rich vascular system with which the ganglion is provided. Its meshes are occupied by the nerve fibres and by *ganglion cells*. These last consist of a substance partly granulous, partly fibrillated, in which a vesicular, spheroidal, sometimes oblong, nucleus is embedded, which itself encloses a shining nucleolus, the position of which may be either central or eccentric.

For the study of these cells, teased preparations must be used. The spinal ganglia of fish, particularly of the roach and pike, the Gasserian ganglion of the frog, or the ganglion through which in the same animal the auditory nerve passes—are best suited for the purpose. If the first are used, the root of the nerve with its ganglion is excised, and macerated in iodized serum, dilute solution of bichromate of potash, or Müller's fluid, for twenty-four hours or more, after which the cells may be teased out with needles. Good teased preparations can also be obtained of the ganglia of the spinal nerves of fish, or frogs in the fresh state. The ganglion cells of fish and frogs, thus isolated, are mostly bipolar, less frequently multipolar. The processes exhibit fibrillar streaking, and, when a process is isolated for some distance, it is found to become invested with a medullary sheath at a short distance from its origin; or, in other words, it assumes the characters of a medullated nerve fibre. The Schwann's sheath of this nerve fibre is continuous with the similar membrane which forms the capsule of the ganglion cells from which it originates, and in which, as in the Schwann's sheath, there are oblong nuclei at regular distances. In the ganglion cells of the Gasserian ganglion of the frog, there are always masses of yellow pigment. In the spinal nerve ganglia of the mammalia, it is only possible to isolate unipolar cells. Good permanent preparations of ganglion cells may be obtained after treatment with chloride of gold. With this view the Gasserian ganglion of the frog, freshly excised and cut into with fine scissors, is placed for ten or fifteen minutes in chloride of gold, and then exposed in slightly acidulated water to daylight, until it assumes a darkish tinge. In preparations of ganglia thus treated and teased in glycerin the ganglion cell substance, along with the axis-cylinder process, is violet red, while the nucleus is pale.*

(*b*) **Ganglion Cells of the Brain and Spinal Cord.**—The spinal cord of the calf or ox are the best objects for this demonstration. The organ must be divided into small portions, which must be placed in bichromate of potash solution, for periods varying from a few days to several weeks. Then a thin slice of grey substance is to be cut with the razor—preferably from the anterior horns—and teased in the liquid in which it has been macerated. Any one who is practised in the use of the needle can also obtain good preparations by teasing from fresh spinal cords, in iodized serum. In preparations of this kind, in addition to the multipolar ganglion cells, medullated nerve fibres, of various diameters, possessed of irregular or regular dilatations (varicosities), and axis-cylinders of various size with distinct fibrillar streaking, are to be met with. In teasing spinal-cord preparations, it is always well to place the glass on a black ground.

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* *Preparation of the Gasserian Ganglion.*—A frog having been rendered ex-sanguine by slitting open the ventricle, the roof of the skull is exposed, and then carefully raised from the occipital region forwards. This process is continued until the internal auricular foramen of the petrous bone can be distinctly seen. Then the medulla oblongata and pons are pushed aside with a needle, and the notch of the pars petrosa cleared of fluid by dabbing it with a fragment of bibulous paper. The fifth nerve is then readily seen. On it, close to where it enters the bone, is a distinctly yellow swelling, which must be carefully exposed by removing the portion of bone which conceals it, and excised with fine scissors.

The ganglion cells of the anterior horns of the spinal cord of the calf are remarkable for their size, and consist of a granular cell substance, in which (as may be seen in preparations in iodized serum under very high powers) fibrils may be distinguished. The large round vesicular nucleus which each cell contains, has a double contour, and encloses a highly refractive nucleolus: in its neighbourhood there is usually a mass of pigment. Each cell possesses processes of two kinds—the so-called axis-cylinder process, and the branched processes. The axis-cylinder process springs from a broad base, from which it tapers to a fine filament. To whatever distance this filament is traced, it is seen that it does not branch, but becomes thicker, and eventually assumes the character of a medullated nerve fibre. The other processes are broad and flattened, and soon divide dendritically. They consist of fibrils embedded in a coarsely granular interstitial substance: the fibrils can be followed distinctly into the ganglion cell. As we shall see subsequently, the terminations of these processes form a dense network of extremely minute filaments, which network is in equally direct continuity with the endings of the nerve fibres which enter the cord by the posterior roots. The cells of the posterior horns are entirely similar, but somewhat smaller. If thin sections of the spinal cord of the pike are hardened in bichromate of potash or chromic acid, washed in water for twenty-four to forty-eight hours, and then placed in diluted ammoniacal solution of carmine for a few hours or a day, good permanent preparations can be obtained by teasing, which can be mounted in glycerin. In the nuclei of Stilling, in the intra-cranial part of the cord, cells occur resembling those of the anterior horn of the spinal cord, and may be prepared in the same way.

Ganglion Cells of the Hemispheres.—If the cortical substance of the mammalian brain be macerated in iodized serum, bichromate of potash, or Müller's fluid, ganglion cells of more or less conical form can be isolated, from the base of each of which several arborescent processes stretch inwards towards the white substance, while the small end of the cone terminates in a process which is simple near its origin, but eventually divides into fine branches, and exhibits everywhere (in iodized serum preparations) fibrillar streaking. Permanent teased preparations may be obtained in the way described above as applicable to the spinal cord.

(*c*) **Ganglion Cells of the Sympathetic System.**—The ganglion cells of the sympathetic system occur either as distinct ganglia of various size (as is seen in the digestive mucous tracts, and in the genital organs), or they are arranged in linear series, or are scattered in greater or less number amongst and between the fibres of nerves. The sympathetic ganglia (as, for example, those of the ganglionic cord or the cœliac ganglion of mammalia) are best studied as follows :—The structure is placed in Müller's fluid or bichromate of potash, and allowed to remain several days until firm enough. Fine sections are then prepared, and teased in glycerin, either at once or after staining in solution of carmine. Another plan consists in steeping sections prepared from frozen ganglia, in chloride of gold for ten or fifteen minutes, and making from them teased preparations, which may be mounted in glycerin.—Again, small fragments of fresh ganglia may be steeped in one-tenth to one and a half per cent. acetic acid, and left in it from twenty-four to forty-eight hours, and then employed in the same way.

The aorta and the bulbus arteriosus of the frog afford excellent preparations. For this purpose the vessel is ligatured at the point of division, and filled with half per cent. solution of chloride of gold by aid of a capillary tube. A second ligature having been placed around the bulb, the part is cut out, and steeped for ten minutes in the same solution. The tube is then opened and exposed, two days or more, in acidulated water, to the light. When of sufficiently dark colour, it is stuck out on a cork with pins. Thin lamellæ may then be stripped off the external aspect of the vessel, spread out on an object-glass, and covered in glycerin.

Meissner's Plexus.—The ganglionic nodules, occurring in the course of the nerves which form Meissner's plexus, in the submucosa of the intestine, may be studied as follows. They are also well seen in longitudinal and cross sections of intestine hardened in chromic acid, and still better in sections parallel with the surface.—Strips of intestine of the cat or dog (after having been washed with half per cent. salt solution, or water coloured slightly with bichromate of potash) are steeped for from forty minutes to an hour in half per cent. gold solution, and then exposed to light in distilled water, and finally hardened in alcohol. Sections are then made in a direction parallel to the serosa, of which of course those only are of use which pass through the submucous tissue. Any one possessed of sufficient dexterity can obtain good preparations by spreading bits of rabbit's intestine, excised and cleansed as above described, on a piece of cork by aid of pins, with the mucous surface uppermost. The mucosa is then worked off as completely as possible with the fine-pointed forceps. Fine flakes of loose tissue must be snipped with the aid of the curved scissors, either from the deep surface of the mucosa, or from the surface from which it has been severed. These are either examined in salt solution in the fresh state, or treated with gold for permanent preparations.

Auerbach's Ganglia.—The ganglia of Auerbach, which are interposed between the transverse and longitudinal muscular layers, are demonstrated as follows :—A portion of fresh intestine of a rabbit or new-born fœtus is blown out with the aid of a glass tube. The operator must then try to strip off with the forceps, from the external surface, a thin membrane, which will be found to contain the serosa and the longitudinal muscular layer. Strips of considerable extent may be thus obtained with a little practice, and must be then treated with gold in the usual manner.

The ganglion cells, which occur in the genital organs, may be best studied in sections or parts hardened in chromic acid, or coloured with gold and then hardened in alcohol.—Good preparations of sympathetic ganglia can be obtained from the bladder of the rabbit. Bits of the fresh bladder are coloured with chloride of gold, and then steeped in acidulated water until they swell out into a gelatinous translucent mass. Thin membranous fragments stripped off with the forceps, or snipped off with the scissors, are spread out and covered in glycerin. To these preparations we shall recur, in connection with the distribution of the nerves among unstriped muscular fibres.

Intimate Structure of the Ganglion Cells of the Sympathetic System.—In each ganglion cell (with the exception of those of the ganglia of Auerbach) the following parts may be distinguished; the capsule, the body of the cell and its nucleus, and the processes. The capsule is beset with strong nuclei at even distances from each other; in sections of fresh ganglia hardened by freezing, and treated with nitrate of silver, markings may be seen in the capsule which indicate the existence of endothelium; the elements of this endothelium are of such size as to make it apparent that each of the nuclei above mentioned belong to an individual cell. As in the ganglia of the spinal nerves, the capsule is continued from the cell upon one of the processes, with the Schwann's sheath of which it becomes identified. The ganglion cells of the sympathetic system are of various size, and are either globular or oblong. In the former case, they may be either without distinguishable processes, or may have a single process (unipolar), or two in opposite directions (bipolar); being in the former case pear-shaped, in the latter spindle-shaped. Others occur which have two processes in the same direction, or numerous processes in various directions (multipolar cells). The substance of the ganglion cell is for the most part finely granular, sometimes containing clumps of pigment of various size. Each cell contains a single vesicular nucleus (or two nuclei), which is usually eccentric, and always contains a large, shining nucleolus. In the examination of a number of ganglion cells, one or two can generally be found in which fine fibrils are distinguishable: these can often be traced nearly to the nucleus, presenting an appearance which seems to correspond with the network of fibres described by some in the body of the cells.

Spiral Fibre Cells.—In the cells of the sympathetic ganglia of the frog, as well as of the cœliac ganglion of mammalia, and in those of the bladder of the rabbit, pear-shaped or club-shaped ganglion cells may be isolated, which possess two processes, extending in the same direction. These processes differ more or less in thickness from each other: they are contained near their origin in a common sheath, which, at a greater distance, divides into two, each investing one of the processes. So long as they are in the common sheath, their arrangement to one another is peculiar. Sometimes they merely cross one another; at others, one of them, usually the thinner, twines spirally round the other. Occasionally, this last is perfectly straight; sometimes it is apparently of the same substance with the body of the cell; at others, it seems to penetrate into its interior, tending towards the nucleus, without, however, being demonstrably united with it. The second process, viz., the so-called spiral fibre, originates by a double or single root, which can be followed to certain nucleolus-like structures, of oblong form, of which from one to four are to be found in the neighbourhood of the pole from which the straight process springs. But whether the spiral fibre is connected with these nuclei, as has been supposed, by a network of extremely fine filaments from which it appears to spring, cannot be determined any more certainly than the question whether, in multipolar cells in general, the processes spring entirely from the substance of the cell, or one or other of them from the nucleus.

Reproduction of Ganglion Cells.—The ganglion cells of the sympathetic system seem to undergo very active development. This appears, first, from the frequency with which cells containing two nuclei are met with; secondly, from the circumstance that frequently two, three, or four polyhedral cells occur in a common capsule; thirdly, from the occasional occurrence of two club-shaped cells in one capsule, so placed that they are in apposition by their flat bases, while their sharp ends are continuous with processes; and finally, that in many organs, as, *e.g.*, in the (male) genital tract, and in Meissner's ganglia of the newly-born fœtus, groups (so-called "nests") of extraordinarily small ganglion cells occur. If the Auerbach's ganglia of the rabbit's intestine are prepared as directed above, and covered in serum, they are found to consist of a network of bands of various breadth, the nodes of which constitute broad plates of irregular form, the whole being invested by a sheathing in which nucleus-like structures are distinguishable. The substance both of the nodes and of the bands which connect them is finely streaked or granular. A greater or less number of ganglion cells mostly globular in form, are embedded in this substance, and arranged either in groups (in the nodes) or in rows (in the bands). In the latter, the chains of cells are interrupted at intervals; the former exhibit numerous perforations, which are merely the interstices of a dense meshwork of bands. In gold preparations, these facts can also be easily demonstrated. In sections of intestine of the rabbit hardened in chromic acid (as we shall see in Part II.), the connection of these ganglia with the ganglionic masses of similar form which exist in the circular fibres, and communicate towards the mucosa with the ganglia of Meissner, can be well seen.

SECTION III.—PERIPHERAL NERVE ENDINGS.

Terminal Organs of Nerve Fibres.—Pacinian Bodies. —The Pacinian bodies are oval or pear-shaped little masses which are found in the subcutaneous tissue of the skin of the finger, and in that of the beak and tongue in birds (goose and duck). In man they are met with also in the genital tract, *e.g.*, in the labia majora, prostate, and corpora cavernosa: in all these situations they can be best studied in sections. They are most easily demonstrated, however, in the mesentery of the cat, in which they are visible to the naked eye as elliptical, transparent bodies, occurring mostly in the fatty parts. Preparations are made as follows:—A mesentery of a cat that has just been killed is spread out on an object-glass and covered with a drop of serum or half per cent. solution of common salt; or a portion of mesentery containing Pacinian bodies is placed in solution of bichromate of potash for twenty-four hours, and then covered in glycerin. We begin our study with the medullated nerve fibre, which enters the corpuscle at one end. From the point at which the nerve parts from the twig from which it is a branch, its course is winding. As it approaches the Pacinian body its sheath becomes thicker, and acquires an appearance as if it consisted of several layers of nucleated membrane. The dark-bordered nerve fibre is separated from the sheath by a distinct, clear interspace, into which oblong nuclei project at regular distances from the internal surface of the sheath, so as to resemble an endothelium. The Pacinian corpuscle may be divided into the neck (the point at which the nerve enters) and the body. In the neck, the lamellæ of the Schwann's sheath split repeatedly, becoming further and further apart from each other, so as to form the well-known concentric capsules of which the body is constituted. Each capsule is beset with regularly arranged flat oblong nuclei; and, in the part of the body which is nearest the neck, each capsule communicates with its neighbours by cross lamellæ, which run obliquely from one to the other. Elsewhere the capsules are discontinuous. In the neck, the nerve fibre is dark-bordered and convoluted, but as it enters the clear space which is contained in the inmost capsule it becomes straight, and at the same time pale and finely streaked. In its course in the axis of this space it is separated from the capsule by a clear interval, into which nuclei arranged at regular distances project. Near the end of the axial space the nerve fibre usually divides into two, occasionally into three, branches, each of which ends in a pear-shaped enlargement (cell), containing a vesicular nucleus. Sometimes the nerve fibre remains undivided, in which case the terminal cell is relatively larger. In the mesentery of the cat I have seen Pacinian corpuscles in which the nerve fibre, instead of terminating, passed out at the end opposite to that at which it entered, eventually ending in another Pacinian body. In this case the relation of the nerve fibre to the concentric capsules, and of these to each other, in the neighbourhood of the point of exit of the nerve, was the same as in the neck. In the most superficial of the concentric capsules, endothelial marking can be seen after treatment with nitrate of silver.

In connection with the Pacinian corpsucles we must mention the so-called "*Endkolben*" (club-shaped endings), which are described in the papillæ of certain mucous membranes, and are said to consist of an axis-cylinder, ending in an enlargement, surrounded by a thickened sheath.

Meissner's Bodies, or Tactile Corpuscles.—These bodies occur in certain broad papillæ of the skin of the volar side of the fingers and of the palm in man. They can be best demonstrated in vertical sections of portions of skin, made across the parallel furrows, and either hardened in chromic acid or in alcohol after treatment with gold. They are oblong bodies, each occupying the axis of a papilla. Their outline is often broken by deep notches. In each corpuscle numerous cross markings are to be seen, which depend partly on the existence of fine fibres, partly on the arrangement of spindle-shaped nuclei. Into each body a medullated nerve fibre, provided with a nucleated Schwann's sheath, finds its way, and then twines once or twice round it: the nerve may often be followed to its upper extremity. Sometimes the fibre appears to enter the corpuscle from one side, in which case it cannot be traced further.

Peripheral Nerve Cells.—Fresh thin portions of human skin (*e.g.*, skin of the prepuce or of amputated extremities), or small portions of the shaven skin of the rabbit's abdomen, are placed for a few minutes in half per cent. acetic acid, and then, after immersion for one or two hours in solution of chloride of gold, are treated in the usual way. In sections of such skin, fine nerve fibres present themselves, which, after penetrating the *rete malpighianum*, are seen to be connected with bodies of an oblong or stellate form, which are strongly stained by gold, and often contain each a distinct, clear, nucleus-like structure. These nerve cells are not really, as has been supposed, terminal organs, for fine fibres are seen not only to reach them, but to pass beyond them, towards the surface. Similar nerve cells exist in the epithelium of the mucous membrane of the mouth and of the vagina. Again, in the network of delicate non-medullated fibres which branch under the epithelium of the tadpole's tail, the nerve fibres are continuous with the processes of branched nerve-cells.

Recently, terminal bodies have been discovered by certain authors in the mucous membrane of the epiglottis, from which it would appear that nerve fibres, either medullated or others, end under the epithelium in club-shaped bodies, consisting of granulous substance, each of which contains one or two nuclei, and is enclosed in a prolongation of the Schwann's sheath of the nerve.—In the mucous membrane of the frog's stomach it is also stated that the nerve fibres end between the cylindrical elements of the epithelium in oval or club-shaped swellings.—Again, in the connective tissue of the bladder of the frog, are to be found cells which consist of a fine granulous protoplasm, and contain several nuclei.—In the skin of the wing of the bat, and in the skin of the ears of mice, the medullated nerves come, at certain parts, into remarkable relation with the papillæ of the hairs. (*See* Chapter XI.)

Peripheral Branching of the Non-Medullated Nerve Fibres in Different Tissues.—Under this head will be described the termination of the nerves in the cornea, conjunctiva, in the tail of the tadpole, in the skin, in certain mucous membranes, in unstriped muscular fibres, in striped muscular fibres, in blood-vessels, and in glands. The nerve endings of organs of special sense will be described hereafter.

Nerves of the Cornea.—In a living or recently killed rabbit, the cornea is excised close to the limbus, and placed in chloride of gold solution for three-quarters of an hour. The preparation is then transferred to distilled water, in which it remains until it has attained a steel-grey colour, the time required varying, according to the season, from six hours to sixteen or twenty. Thence the object is transferred to a small wide-mouthed vessel, which contains a small quantity of nearly-concentrated, filtered solution of tartaric acid. As soon as it has had time to absorb the liquid, its colour becomes deeper, and changes to greyish-violet. If the bottle is then plunged into water at a temperature of 40° to 50° C, to such a depth that both liquids stand at the same level, the preparation assumes, after a few minutes, an intense violet-red colour, which goes on increasing until it attains a dirty brownish-red, and exhibits a velvety lustre. The cornea is now removed, and steeped in distilled water for two hours or more. The epithelium, along with a thin layer of corneal substance, is then stripped off with the aid of the pointed forceps, beginning from the sclerotic beyond the edge. In a preparation thus obtained, it is seen that there exists in the anterior, *i.e.*, most superficial, layer of the *cornea propria*, a plexus of nerves of various breadth; each of these nerves consists of a bundle of minute fibrils, invested in a pale Schwann's sheath with oblong nuclei, within which they may either run parallel to each other, or wind round each other in a more or less spiral manner. Wherever a bifurcation occurs, or two nerves join, there is an enlargement, in which the individual fibrils are distinctly woven together into a network. From the nerves of this plexus, fibrils are given off either alone or in tufts. Corneas prepared in the manner above described may be also advantageously employed for the preparation of vertical and horizontal sections.

The nerves of the *substantia propria* of the cornea of the frog are best demonstrated as follows:—A silk thread having been passed through the centre of the cornea of *rana esculenta* and brought out again at the sclerotic ring, the two ends are knotted together. After the thread has remained from five to eight hours, the cornea is excised and placed for twenty minutes or more in half per cent. solution of chloride of gold. It is then transferred to distilled water, and exposed to light until it acquires a dark violet red, or reddish-brown colour; the time required for this purpose varying from one to three days, according to the season. The epithelium must now be removed with the aid of sharp-pointed forceps, along with a very thin layer of corneal tissue, after which the cornea is to be mounted in glycerin. In such a preparation it is seen that the nerve trunks form a rich plexus by division and anastomosis in the corneal substance. The branches of this plexus may be distinguished as nerves or bundles of the first order, and resemble in their structure the corresponding nerves already described in the cornea of the rabbit. From these, smaller bundles, not possessed of a nucleated sheath (nerves of the second order) are given off. These run a course which is sometimes winding, sometimes straight, and are connected by scanty anastomoses, so as to form a plexus of large meshes. They give off, either laterally or terminally, the fibrils of the third order, which have the following characters:—Their general direction is towards the surface. In taking this course they divide into finer and finer filaments, so that the finest are scarcely distinguishable under the highest powers, and form, by repeated anastomoses, a network which lies immediately under the epithelium. The fibrils themselves are beset with minute granular varicosities. In either case these form a network, the meshes of which are oblong or quadrangular.

Nerves of the Conjunctiva and Membrana Nictitans.—For the study of the nerves of the mammalian conjunctiva, the *conjunctiva fornicis,* or the *plica semilunaris* of the eye of the pig, calf, or rabbit answers best. Portions of the fresh plica semilunaris are treated in the same way as the cornea of the rabbit. As soon as the proper degree of coloration is attained, the preparation is hardened in diluted alcohol. Sections are then made in both directions, and covered in glycerin. The conjunctiva fornicis is prepared free over a considerable surface, and spread out on a cork with the free surface upwards. It may then be immersed in gold solution in a capsule, or this liquid may be poured on it, after which it must be treated as before. The demonstration of the fine nerves of the conjunctiva is not so easy as of those of the cornea, so that a general idea of their distribution can only be obtained by a comparison of a number of preparations. The nerve trunks, composed of medullated fibres, divide in the superficial layer of the mucosa into small branches, each containing two or three medullated fibres, in which varicosities occur here and there. These mostly accompany blood-vessels, following a winding course, and, by their communications, forming a plexus. They give off under the epithelium non-medullated fibres, by the anastomosis of which a scanty sub-epithelial network is formed. I have seen fibres originating from this network making their way towards the surface among the epithelium cells, and dividing dichotomously, but have been unable to trace their further course.

The mode of preparing the membrana nictitans is the same as that for the cornea. The fresh membrane is placed for twenty minutes in chloride of gold, and then in distilled water until it is of a dark colour. The epithelium of the anterior surface is then stripped off with the sharp-pointed forceps, after which the preparation is covered in glycerin. The objects which present themselves are (1) The flask-shaped glands (with their short, narrow ducts), lined with spheroidal, granular, nucleated cells; they are surrounded by a layer of spindle-shaped cells, not unlike muscle-cells. (2) Granular, large, flat, branched cells, brightly stained, possessing oblong, flat nuclei, and sending out processes which communicate in the same way as cornea corpuscles. (3) Pigment cells, some of which are much branched and communicate with each other, while others are isolated and clump-shaped. (4) A rich network of blood-vessels. (5) Nerves. From the plexus of medullated nerves, separate medullated nerve fibres spring, which, close to their origin, lose their medullary sheaths. The non-medullated fibres possess numerous oblong nuclei.

Nerves of the Skin.—We have already had occasion to describe the method of preparation to be adopted for the study of the nerves in the skin. It may, however, be well to add that, immediately after removing the preparation from the gold solution, it is possible to cut sections. The nerve trunks, which find their way from the subcutaneous tissue towards the epidermis, unite at the surface of the corium to form a dense network of non-medullated fibres, from which fine fibrils stretch vertically into the *rete Malpighii*, either as isolated fibrils which pass up into the epithelium between two neighbouring papillæ, or as groups of several fibrils which pierce the tips of the papillæ. In the *rete Malpighii* the nerve fibres often divide, and occasionally communicate with their neighbours by horizontal branches, or with processes of the deeply stained branched cells above described. Isolated fibres may be traced in the *rete Malpighii* to within a short distance of the horny layer, where they either seem to lose themselves in swellings of various size, or to divide dichotomously beyond; eventually returning towards the corium. —The hair bulbs are also surrounded by a network of fine non-medullated nerve fibres, the further description of which will be found in another part.

Nerves of the Tadpole's Tail.—The best object for the purpose is the tadpole of *Hyla*. The distribution to be now described may be studied in recent preparations in half per cent. salt solution or serum. It is, however, better to make preparations by the method fully described in the chapter on connective tissue. The peripheral nerves of the tadpole's tail are derived from a plexus which lies immediately underneath the sub-epithelial hyaline layer; the nerves which form it are composed almost entirely of non-medullated fibres, which are invested in a sheath beset with oblong nuclei. From this plexus similar fibres arise towards the epithelium, and by division become smaller and smaller, anastomosing with each other so as to form a second plexus nearer the epithelium than the other. They also possess nuclei, the position of which in relation to the fibre is sometimes lateral, sometimes apparently axial. In the more superficial plexus, spindle-shaped enlargements are frequently seen at equal distances from each other. These are distinctly granular, and each contains one oblong, clear, sharply-defined nucleus, and nucleoli. They are to be considered as bipolar ganglion cells, occurring in the course of the fine non-medullated fibres.—Immediately under the epithelium the densest branching of the fine, pale fibres is seen. These bifurcate repeatedly, displaying at tolerably regular distances, and especially at the points of division, numerous granular swellings. The branchlets arising from this repeated division join each other archwise, forming a very close network, the meshes of which are round, or more often polyhedral, and of such size that two or four of them can be covered by the nucleus of an epithelial cell. In this network, nuclei and cells are scattered, the former being sharply defined and of oblong or irregular shape, exactly similar to those mentioned above as occurring in the fine non-medullated fibres. The cells are spindle- or (more frequently) star-shaped, flat, and finely granular, each containing a roundish nucleus. Their short pointed processes are in continuity with the fibres of the nerve plexus. They may be regarded as multipolar ganglion cells. (*See* p. 77, "peripheral nerve cells.") I could never find any connection between the pale nerve fibres and the well-known pale or pigmented branched cells of the connective tissue.—From these facts we learn that the fine nerves of the tadpole's tail terminate close to the epithelium in a dense network of pale fibres, extending equally on both sides of the tail so as to form a continuous sub-epithelial layer. As no nerve fibres can be traced beyond this network, we are entitled to conclude that the nerves terminate in it.

In preparations made in the same manner from the intestine of a nearly mature human embryo, it is possible to make out that, in the reticular system above described, numerous small cellular structures occur, embedded in the substance both of the bands and nodes, in most of which all that can be seen is a nucleus surrounded by a very narrow *entourage* of granular substance: a few present the ordinary characters of ganglion cells.

Nerves of the Mucous and Serous Membranes.—Thin strips of the fresh mucous membrane are cut from the vagina or mouth of the dog or rabbit, and are placed for from forty-five to sixty minutes in half per cent. solution of chloride of gold, and are then, after having been washed with distilled water, transferred to a solution of tartaric acid, hardened in alcohol, and employed for the preparation of sections in the manner already explained. The nervous trunks which are distributed to the mucous membrane consist mostly of medullated fibres, and give off branches which resolve themselves into a network of fine non-medullated fibres, lying immediately beneath the epithelium, and the films of this network are beset with nuclei, which are either far apart, as in the vaginal mucous membrane of the dog, and in the oral and vaginal mucous membrane of the rabbit, or more frequent, as in the mouth of the dog. From this network, filaments having varicosities of various size, find their way into the epithelium, and give off branches to the different layers of epithelium which combine into a network, some of which appear to end in a knob-like swelling. In the middle layers they are in communication with branched nerve-cells: there is no evidence of any connection between them and the branched cells of the mucosa.

Nerves of the Septum Cisternæ and of the Mesentery of the Frog or Newt.—It is comparatively difficult to demonstrate non-medullated nerves in these parts by means of the ordinary method of staining with gold. It can be done in the following way successfully:—The fresh membrane is placed for from forty-five to sixty minutes in solution of gold; thereupon it is exposed to the light for several days in distinctly acid water. As soon as the preparation has acquired a markedly reddish or greyish violet tint, it is pencilled on both sides so as to remove the endothelium, and placed for sixty minutes in diluted, distinctly alkaline solution of carmine. It is then washed in acidulated water and covered in glycerin. From the winding nervous trunks which accompany the larger vessels of the mesentery, numerous small twigs branch off in great numbers, consisting of very numerous non-medullated fibres, which combine to form a network. Although these fibrils are much more numerous than has been hitherto supposed, they never terminate by a free end, but always take part in the formation of a network. (The numerous non-medullated fibres which are distributed to the blood-vessels will be described elsewhere.)

Nerves of the Peritonæum.—For the demonstration of the fine fibres of the peritonæum of the rabbit, the following is the best method:—Three drops of concentrated acetic acid are added to twenty cubic centimeters of distilled water. To this mixture five drops of half per cent. solution of gold is added. The fresh peritonæum is immersed in the solution, and allowed to remain exposed to the light for several days until it becomes darkly stained. Very instructive preparations may be obtained by preparing in the same way the fold of the peritonæum, which stretches backwards and to the left, from the diaphragm to the upper surface of the stomach, close to the cardia.

Nerves of Unstriped Muscular Fibres.—The bladder of the frog, the small arteries of the same animal, the muscular coats of the intestine, or of the vagina of the rabbit, may be employed. The following methods are applicable:—As regards the bladder of the frog, the previously described method of preparing the muscular fibres themselves, also serves for the demonstration of their nerves. In the bladder of mammalia, the mixture of acetic acid and gold, mentioned above in relation to the preparation of the nerves of the peritonæum, answers well. After the preparation is sufficiently stained, thin shreds of muscular tissue are stripped from the external surface of the swollen membrane, and prepared in glycerin. In the large arteries of the mesentery of the frog, the method already employed for the demonstration of the non-medullated nerve fibres of the mesentery generally, is to be used.—The relatively large arteries of the frog (as, *e.g.*, those of the root of the mesentery) can, as a rule, be advantageously prepared by placing them for five minutes in half or one per cent. acetic acid, and then either allowing them to stand in the gold solution twenty to thirty minutes, or transferring them to chromic acid solution of one-tenth per cent. for from thirty minutes to an hour. For the unstriped muscular fibres of the intestine, uterus, &c., sections of frozen organs may be treated with acetic acid and gold, or chromic acid, in the same way. Finally, small portions of the same tissues may be steeped in gold solution, washed in distilled water, treated with tartaric acid, hardened in alcohol, and employed for the preparation of sections. The facts thus demonstrated may be summed up as follows:—Nerve trunks of various size run in the sheaths of connective tissue which lie between the muscular bundles. These trunks consist either of non-medullated fibres, or of medullated, or of both kinds mixed, and form a plexus with wide meshes. In this (which may be termed the *principal plexus*) the ganglion cells which have been already described are intercalated. Its nerves give off numerous fibres, some of which are medullated, but soon lose the medullary sheath, others non-medullated. These last are pale, streaked longitudinally, and have nucleated sheaths. By their abundant ramifications, they form a network of rhomboidal or oblong meshes, having nuclei at their points of junction. This network involves the individual muscular bundles, and is called the *intermediary network*. Fine filaments, containing granules, spring from it, which penetrate between the muscular cells, and divide dichotomously in this situation, forming by their connection the *intra-muscular network.* In addition to the fibrils which lie between the fibres, the network contains others, which penetrate the muscle-cells and become connected with the nucleoli of their nuclei, in such a way, however, that the nucleolus is not the end of the fibril, but is intercalated in it. It is only in a few, out of a great many successful preparations, that the intra-muscular network can be demonstrated. Most serve to show only the intermediary plexus.

Nerves of the Striped Muscles.—The demonstration of the nerves of voluntary muscle has, hitherto, been accomplished only in fresh preparations; there are, however, one or two cases in which the silver method can be used. It is, in the first place, to be borne in mind that only muscles that are still irritable are of any use for the purpose. Secondly, that the greatest care must be taken in making the preparation, especially to prevent the cover-glass from pressing, by strips of paper.

Muscular Nerve Endings of the Water-beetle.—The muscles of certain invertebrate animals, *e.g.*, *Dytiscus*, or, still better, *Hydrophilus piceus*, and particularly those which pass from the thorax to the legs, are best suited for the purpose. The muscle is severed near its insertion with fine, sharp scissors, and at once placed on the object-glass and covered, or transferred to a drop of serum and spread out so as to separate a few muscular fibres. It is easy to recognize the broad, riband-shaped, medullated nerve fibres, each possessing a striated axis-cylinder, which rapidly divide into finer non-medullated fibres, each distinctly streaked and beset with nuclei. A single muscular fibre may receive several non-medullated nerve fibres. At the point at which each enters the muscular substance, a more or less marked elevation is distinguishable, the so-called *Doyère's prominence.* This consists of granular substance in which clear, roundish nuclei are embedded. The prominence, with its nuclei, is lengthened out into processes in directions corresponding with that of the axis of the muscular fibre. These processes may either stretch along the surface of the muscular fibre, or sink into its depth. Sometimes the prominence is represented by a mere lamina of granular substance, which does not project above the surface. The axis-cylinder penetrates into the substance of the prominence, passing through the sarcolemma, with which its Schwann's sheath becomes continuous. It usually divides dichotomously in the prominence, each branch ending in a rounded extremity. The prominence, therefore, consists of two parts, viz., the axis-cylinder, with its two branches, and the nucleated granular substance in which it is embedded. The granular substance consists, in all probability, of the same material as that which constitutes the so-called muscle-corpuscles.

Muscular Nerve Endings of the Frog.—In many respects the nerves of the muscle of the frog differ from those above described. In the first place, there are many muscular fibres which are entered by only one nerve. In order to make out this fact, it is a good plan to place portions of muscle in a mixture of chlorate of potash and nitric acid at 40° C.; or, better, to place the tissue for twenty-four hours or more in diluted sulphurous acid, after which it is exposed, still remaining in the liquid, to a temperature of 40° for a few hours. If the muscle is then shaken with water in a test tube, the individual fibres separate very readily from each other, and may be covered without further preparation.—For the study of the finer relation of the muscular nerves, separate fasciculi of the gastrocnemius may be employed, which must be cut out with their tendons—those parts being chosen to which vessels and nerves can be traced with the naked eye. The preparation is covered in *humor aqueus,* after it has been spread out with great care with needles. It is then possible to observe, that a medullated fibre comes into contact here and there with a muscular fibre, and divides into several medullated branches. Just as the branches approach the point at which they enter the sarcolemma, in order to attain the surface of the muscular substance, they lose their medullary sheath. At this point, they resolve themselves into a number of small pale filaments, which run parallel to the long axis of the muscle, keeping close to its surface, and are beset with oblong structures resembling nuclei. Eventually, each terminates abruptly in a rounded end.

Another excellent object for demonstration of the muscular nerves is the thoracic cutaneous muscle of the frog, which must be divided along its insertions, and then severed from its thoracic attachments, and carefully spread out in a drop of *humor aqueus* and covered, care being taken to interpose srips of paper underneath the edge of the cover-glass. It is also possible to demonstrate the nerve endings in frog-muscles with the aid of nitrate of silver—the same parts being used for the purpose. The isolated fasiculi are placed in serum, to which an equal quantity of distilled water has been added, for ten or fifteen minutes. Thence they are transferred to a quarter per cent. solution of nitrate of silver for thirty or sixty seconds, and then exposed to the light until they acquire a brownish colour. They are further prepared in a drop of a mixture of equal parts of ordinary acetic acid, glycerin, and water. In such preparations a system of clear lines shows itself in the striped brown ground of muscular substance. These lines correspond exactly, in their whole arrangement, with the intra-muscular nerves above described.

Muscular Nerve Endings of Snakes and Lizards.—The most beautiful muscular nerve endings with which we are acquainted are those of the reptilia, *e.g.*, *Lacerta agilis*, *Lacerta viridis* and *Coluber natrix*. In preparations of the muscle of the thigh or of the back of the lizard in *humor aqueus* or serum, it is seen that the medullated nerve fibres divide into branches, in the same way as in the frog. Here, as before, the branches lose their medullary sheath just as they enter the sarcolemma, and then resolve themselves into a beautiful digitate or fringe-like expansion of pale fibres embedded in a granular ground containing nuclei, resembling that described in *Hydrophilus*, but of a laminar form. In the subcutaneous muscles of *Coluber natrix*, the terminal expansion forms a rich network of riband-shaped fibres embedded in a granular ground. The network is so close that it looks like a lamina in which round and oval orifices have been punched out. In silver preparations made as above directed, as well in the lizard as in the snake, the same facts may be demonstrated—the intra-muscular system of nerves exhibiting themselves as clear lines on a brown ground.

The endings of the muscular nerves of mammals resemble those of reptiles.

From the preceding details it appears that two forms of muscular nerve endings may be distinguished. In the first form, the ends of the axis-cylinder, or those of its branches, lie in immediate contact with the muscular substance underneath the sarcolemma (frog). In the second, they are embedded in a granulous ground (*Hydrophilus*, reptilia, mammalia). The demonstration of nerve endings is one of the most difficult tasks which can be undertaken by the histologist.

PART II.

PREPARATION OF THE COMPOUND TISSUES.

CHAPTER VI.

METHODS.

THE methods of examining tissues in the fresh state, with or without the addition of reagents, and of isolating the elements by the process of teasing with needles, have been fully described in the First Part. We have also seen that, in transparent structures, particularly membranes, the anatomical relations of the elements may be studied, either by observing them in the natural condition, or after preparation with the solution of chloride of gold, or with that of nitrate of silver. For the investigation of the compact tissues, other modes of preparation are necessary, in order to bring them into such a condition that fine sections can be made of them. It is the purpose of this chapter to describe the method by which this is accomplished.

Preparation of Sections of Fresh Tissues.—There are a few organs or parts of organs which possess such a consistence, that it is possible, without preparation, to make microscopical sections of them; such as cartilage, some tumours, skin, hypertrophied lymphatic glands, prostate gland, kidney, liver, and under certain circumstances involuntary muscle. Sections of these tissues serve either for the study of the condition of the elements, or the action of reagents; or are made with a view to treating them with gold or silver. They are, however, mainly useful as facilitating the preparation of the individual elements by the process of teasing. For this purpose the section may be either used in the fresh state with indifferent liquids, or after maceration in iodized serum, Müller's fluid, or one per cent. solution of bichromate of potash.

For the study of the anatomical relation of fresh tissues, other methods must be used. The simplest plan is to take the object in the hand, and use a sharp section knife. It is sometimes recommended to fix the tissue between elder pith or cork, by mechanical means. This is not advantageous on the following grounds:—Those tissues which are soft are so injured by the pressure that their elements are in a completely unnatural condition; whereas, in the case of firm tissues, it is quite easy to do without such assistance.

Preparation of Sections by Freezing.—For the purpose of obtaining sections of tissue without any dislocation or alteration of structure, the method of freezing is well adapted. A freezing mixture is prepared by introducing alternately small quantities of broken ice, or snow (not so advantageous), and of finely powdered salt, into a large vessel, mixing the two ingredients thoroughly after each addition. The temperature should be determined by the introduction of a thermometer. The object, which must be small, should be cut to an oblong form, and placed on a flat cork, much wider than itself. It must be pinned to this cork at the end opposite that from which the sections are to be cut. In the case of a membrane, the object must be folded, and fixed in the same way. The whole is then placed in a platinum crucible, which has been previously plunged into the freezing mixture. The crucible must be at once covered, and a little of the freezing mixture placed on the top of it. The section knife, which must be sharp, is cooled by laying it on ice. As soon as it is ascertained, by exploration with a needle, that the preparation is firm enough, the knife is handed to an assistant, who wipes it, and holds it in readiness. The cork is then taken out with the forceps, and seized by the fingers of the left hand in such a way that they do not come into contact with the preparation. A succession of sections having been rapidly made, the number varying with the skill of the operator, the cork is replaced in the crucible. The sections may be employed either for immediate examination, or for teasing, or subjected to further processes of preparation. As soon as the portion of tissue in the crucible is again of the proper consistence, more sections can be made. As regards the temperature which should be employed, and the time during which the object should be frozen, no definite rule can be given. It may be stated, in general, that temperatures varying from 6° to 20° C. are sufficient for all purposes. The time necessary for the attainment of the proper degree of firmness is obviously dependent on the temperature of the freezing mixture, on the thickness of the object, and on the relative quantity of water it contains. Accordingly the time is very variable, so that the proper moment for removing the preparation can only be determined by frequently repeated exploration; by which means alone it is possible to avoid the risk of carrying the hardening too far—a result which is alike prejudicial to the structure of the organ, and to the success of the section.

Methods by which Tissues are Hardened for the Preparation of Sections.—For the purpose of rapidly hardening tissues, small portions may be advantageously placed in chloride of gold, osmic acid, or chloride of palladium, and kept till they are sufficiently consistent. Such preparations must usually be embedded in the manner to be hereafter described, before sections are made from them. The sections themselves are then exposed to the light in distilled water, and covered in glycerin. Half per cent. solution of chloride of gold, solutions of perosmic acid varying from one-tenth to two per cent., or solutions of chloride of palladium from one-tenth to half per cent., are used.

Other agents and methods in use are the following:—Alcohol, oxalic acid, boiling and drying, chromic acid and its compounds. (*a*) For thin membranous tissues, hardening in alcohol answers well. It is more rapid than chromic acid, which, however, has superseded it for many purposes for which it was formerly employed. Absolute alcohol is used principally for hardening brain, and for injected tissues. Common alcohol is also used for the hardening of pancreas, salivary glands, and the glands of the stomach and intestine, and of objects which have been already treated with gold or silver. Further, when tissues have been partly hardened in chromic acid compounds, the hardening can be accelerated and completed by subsequent immersion in common alcohol. (*b*) The use of oxalic acid and oxalates, and other similar salts, may be entirely dispensed with. If used, weak solutions of from a half to two per cent. are preferable. (*c*) The process of boiling, &c., is entirely relinquished. In former times it was employed for intestine, kidney, trachea, and larynx. The intestine was boiled in a mixture of water, creosote, and vinegar, stretched on cork, and dried. Sections were made with scalpels, and then steeped in acetic acid. (*d*) The chromium compounds are the most valuable agents we possess for hardening—viz., chromic acid, in solutions varying in strength from one-tenth to half per cent.; bichromate of potash, in solutions of from half to two per cent., and Müller's liquid, which consists of two parts of bichromate, and one part of sulphate of soda, in 100 parts of water. These have the immense advantage that they produce no marked shrinking or distortion of the tissues, so that they retain for the most part their natural characters. This is particularly the case as regards bichromate of potash and Müller's liquid. Very small portions of tissue must be used, particularly when chromic acid is employed, for it penetrates much less readily into the tissues than the others; so that if the preparation is too large, it is apt to become putrid in the centre, while the outside is too hard. If the objects are smeared with foreign matters, as, *e.g.*, intestine by intestinal contents, blood or mucus, it is desirable to rinse them in water coloured yellow by bichromate of potash, before introducing them into the hardening liquid. The quantity of liquid must be large in proportion to the size of the object. If the process does not go on quickly enough, the liquid must be renewed. Chromic acid hardens much more readily than bichromate or Müller's liquid, from two to five days being often enough for the former, while as many weeks are required for the latter. Its greatest disadvantage is that the tissue becomes brittle if it is left in it beyond the time that is necessary. It is, on this account, a good plan to transfer the objects to common alcohol before they have acquired the requisite consistence. The alcohol not only serves to complete the hardening, but to preserve the objects in a state fit for use. For some tissues, chromic acid is not suitable to begin with, *e.g.*, retina, ovary, or kidneys. For all these organs, the bichromate of potash must be used. After two or three weeks they are transferred to chromic acid or alcohol, to complete the hardening.

Embedding.—It has been several times mentioned that small portions of hardened tissues must be embedded. This is effected by immersing the bits in a fluid mass, which can be rendered solid either by cooling it or depriving it of water; the purpose being, first, to render it possible to hold the bit, and secondly, to facilitate the cutting of sections equally thin throughout. Mixtures are used of stearin and oil, stearin and wax, paraffin and oil, paraffin and wax, paraffin spermaceti and oil, wax and oil, gum arabic, gelatin, gelatin and glycerin. Among the fatty mixtures, the best, cheapest, and easiest to prepare, is wax and oil. Next comes the mixture of paraffin spermaceti and oil. For portions of tissue which have an uneven surface, especially if the inequalities are close together, embedding in gelatin or gum is more to be recommended, especially to those who have not had much practice.

Embedding in Wax and Oil.—For this purpose pure white wax and pure olive-oil should be used. Equal quantities of these ingredients are warmed in a capsule till all the wax is fused; they are then thoroughly mixed with a glass rod. It is better to prepare a considerable quantity at a time, although only very little is required for one embedding. The proportion of wax to oil depends on the consistence of the object to be embedded; the more wax being employed the firmer the object, and *vice versâ*. When sections of compact tissues (*e.g.*, glands of the organs of digestion, trachea, larynx and muscle, bone, the eye and its appendages) are to be made, the mode of procedure is as follows:—If the organ has been hardened in alcohol, an oblong bit must be cut from it with a razor, including the part of which it is desired to make sections. If it has been hardened in any aqueous solution, *e.g.*, chromic acid or bichromate of potash, it must be first steeped in common alcohol. According to the size of the bit, a little box or case, of paper or any suitable material, such, for example, as zincfoil, must be made, so that it will hold the fused mixture. When paper is used, the sides are joined with gum or paste, or are merely pinned together. The box should be about half as long again as the object used. When ready, it is filled with the fused wax-mass to a depth sufficient to cover the object. As soon as the mass begins to solidify at the sides, the bit is introduced as follows:—A needle is stuck slightly into the end opposite to that from which sections are to be cut, and the bit is plunged into the mass with its long diameter horizontal, and in such a position that the end furthest from the needle is near, but not in contact with, the side of the box, and, consequently, the other end is at a considerable distance from the side. In this way, although the whole is surrounded with the wax-mass, there is a greater thickness around the end into which the needle is stuck, so that the whole can be securely and conveniently held. The solidification can be accelerated by immersion in water or alcohol. If the portions of tissue are compact enough, it is possible to perforate the bit with a very slender needle, the point of which is stuck into the table or cork on which the box rests; by this means the operator is saved the trouble of holding the needle till the wax-mixture solidifies. In finally withdrawing the needle, the greatest care must be taken to give it a twisting motion, as otherwise, especially if the object is thin, it is apt to be displaced. If the object contains a cavity communicating with the surface by a single opening (*e.g.*, the cochlea), it is necessary first to fill the cavity with the mass: this is done either by placing it *in vacuo*, or by making an additional opening. If a thin membrane is to be embedded, of such tenuity that a needle could not be introduced without danger of destroying it, the following methods may be used:—(1) A box is half filled with the mass, and then, as soon as it begins to solidify, the membrane is applied to the half-solid surface, in such a position as is most suitable with reference to the direction in which the section is to be made. The box is then filled with a thoroughly fused mass, care being taken that it is not too hot. (2) The fused mass is allowed to drop on an object-glass or a thin flat piece of cork, so as to form a layer thick enough to serve as a basis for the object, which is then laid upon it and covered with an additional layer of wax-mass. If an object-glass is used, it must be first covered with turpentine, otherwise it will be difficult to remove the solidified mass from it. In all cases the surface of the object must be nearly dried before embedding, otherwise the mass will not adhere to it.

As regards the other fatty masses, the only one which can be recommended is a mixture of five parts paraffin, two parts spermaceti, and one of lard. It is, however, decidedly inferior to the mass of wax and oil.

Embedding in Gum or Gelatin.—It has already been stated that objects with delicate projections in close proximity to each other (*e.g.*, papillæ or villi), can be better embedded in gum or gelatin than in wax and oil. The wax-mass, in solidifying, does not penetrate between the projecting parts, so that they are unsupported, and consequently are apt to be broken off in making sections. Gum is solidified by immersion in alcohol, gelatin by cooling: in both cases the process is so slow that the mass has time to penetrate between the inequalities of the surface of the object. The gum or gelatin solution must be concentrated; to the gelatin a little glycerin should be added. I think gum preferable, first, because the consistence of the solid mass can be varied according to the time it is left in alcohol; and even if it has already become too hard, it may be softened by adding to the alcohol a few drops of water. No such modification is possible in the case of gelatin. It is also more easy to make sections in gum than in gelatin, the elasticity of which is a great disadvantage. On the other hand, it is easier to embed in gelatin, and the time required for solidification is much shorter. The method of embedding in gum is as follows:—A thick solution of powdered and sifted gum arabic is prepared in a beaker, and allowed to stand in a water-bath until all air-bubbles have collected at the surface in the scum, which must then be removed by skimming; after which the solution may be used. A little box of paper is then prepared, of suitable size, which is placed on a plate of cork. The bit to be embedded is then stuck through with a needle, the point of which is thrust into the cork through the bottom of the box; the same rules being followed as regards the position of the bit in the box as in embedding in wax-mass. The whole is then transferred to a glass capsule. As soon as the bit is nearly dry at the surface, the solution is poured along a glass rod into the box until it is full to the brim. Alcohol is then carefully poured into the capsule, until the little box is immersed to half its height. The whole must then be covered over and left for two or more hours. As soon as the gum becomes opaque and white on the surface, which occurs in about the time mentioned, the whole mass can be immersed in alcohol until it is brought to the required degree of solidity. The process may be accelerated either by changing the alcohol frequently, or by using absolute alcohol. If the mass is too hard, it can be softened by adding a drop or two of water to the alcohol, as has been already stated. When gelatin is used, the mode of procedure, so far as relates to the preparation of the solution, is similar. The bit having been fixed into the box and surrounded with the solution, the whole is allowed to stand until it becomes solid. Whichever material is used, the mass is freed from the paper box as soon as it has acquired sufficient firmness, and the ends of the needle are snipped off above and below.

Preparation of Sections of Hardened Tissues.—For making sections, razors are most used. Other instruments are also employed, the purpose of which is to make up for want of skill in the operator. The principal ones are Valentin's knife, the microtome of Hensen, that of His, another microtome lately described by Brandt, and the section cutter of Stirling, lately improved by Rutherford. Of these, the most useful is that of His, which has the advantage that it is possible to cut with it successive sections of an organ in equidistant planes, parallel to each other, with the greatest exactitude.

The razor or section knife, in the hands of a skilful operator, is superior to any of these contrivances. The knife I use is of the form shown in Fig. 16. The blade measures eight inches; the wooden handle is massive, so that it can be firmly grasped. One side of it is flat, the other slightly concave, it is thus extremely thin to a considerable distance from the cutting edge. When sections are to be made of objects embedded in wax-mass, the knife must be wetted with common alcohol, in which liquid each section must be immersed as soon as it is made. Sections of objects which have been embedded in gum or gelatin must be placed in water, but the knife wetted with alcohol.

Colouring of the Sections.—It is quite unnecessary to refer to all the coloured liquids which have been used for staining. It will be sufficient to describe the mode of using carmine and anilin.

Carmine.—The most simple solution for the purpose is the following:—Two grammes of carmine in fine powder are thoroughly mixed in a beaker, with a few drops of water. Four cubic centimeters of liquor ammoniæ are then added, and forty-eight cubic centimeters of distilled water. The liquid is filtered into the stoppered-bottle, in which it is to be kept. The bottle is then left open for a few days, in order to get rid of the excess of ammonia. One or two drops of this solution are introduced into a watch-glass, and diluted with distilled water to such an extent, that when it is placed on a written or printed sheet of paper, the letters can only just be distinguished through it. The sections are immersed in the diluted liquid till, on inspection, they appear to have the tint desired. Prolonged steeping in dilute solution gives, as a rule, better results than rapid staining in strong solution; for, in the former case, although the colour is less intense, the different tissues are rendered distinct by the different degrees to which they are stained. I use the carmine solution for this purpose as follows :—The sections having been allowed to remain for twenty or twenty-four hours in a liquid consisting of one part of carmine solution and nine-twelfth parts of distilled water, are washed for a short time in distilled water, and transferred either to glycerin (if it is intended to mount them in this medium), or to alcohol (if they are to be mounted in Dammar). If the sections have not been previously in alcohol, it promotes the staining to put them for a few minutes into that liquid. If it is intended to preserve the sections in glycerin, it is desirable to add a few drops of it to the staining liquid. The well-known liquid used by Beale for staining fresh tissues may be also employed for staining sections; but, in preparing it for this purpose, the alcohol may be omitted. The composition of Beale's liquid is as follows :—

Beale's Solution.—Ten grains of carmine are heated in half a drachm of liquor ammoniæ. As soon as the liquid is cold, two ounces of distilled water, two ounces of pure glycerin, and half an ounce of alcohol are added. The solution is then either filtered or decanted from the undissolved carmine. This liquid requires no dilution. A small quantity must be warmed in a watch-glass to get rid of the ammonia, and it is then ready for use. We shall find that, in the preparation of the mucous membrane of the stomach, it is of special value.

Anilin.—Anilin is used in aqueous and alcoholic solution; the former being most useful. It is obtained by treating anilin blue with sulphuric acid. Two centigrammes of the soluble product are dissolved in twenty-five centimeters of distilled water, and twenty to twenty-five drops of alcohol. This solution colours sections which have been in alcohol very rapidly.

Picric Acid is used in very dilute solution for the purpose of staining sections yellow. Sections may be first stained in picric acid, then in carmine, in which case the muscles are coloured yellow. Whatever the staining liquid employed, the sections must be transferred, as soon as they are sufficiently coloured, to distilled water with or without the addition of a trace of acid.

Methods of Mounting Sections.—Sections may be covered either in glycerin, in mixtures of gelatin and glycerin, of glycerin and acetic acid, of glycerin acetic acid and alcohol, in Canada balsam, or in Dammar varnish. If glycerin is to be used, the sections should, if they have been in alcohol, be previously placed in water. Glycerin alone, answers best for sections of tissues treated with gold or silver. Sections of organs treated with osmic acid must be placed in acetate of potash. Very thin unstained sections of glandular organs and of connective tissue may be temporarily mounted in glycerin, but cannot be preserved for a length of time in that liquid.

All sections which are intended to be permanent, excepting those of tissues prepared by the gold or silver methods, must be mounted in Canada balsam or Dammar; the last being preferable, as more easy to manipulate. It is prepared as follows :—

Preparation of Dammar Varnish.—Half an ounce of gum Dammar in powder, is dissolved in an ounce and a half or two ounces of turpentine, and half an ounce of gum mastic in two ounces of chloroform. The two solutions are then separately filtered and mixed. This varnish so obtained is clear, and if exposed in a thin layer on a plate of glass solidifies rapidly. The sections which are to be mounted must be placed, for a quarter of an hour or more, in a capsule containing absolute alcohol, which should be provided with a cover. Each section must be raised with the aid of a german-silver or copper lifter (the blade of which is then placed on blotting-paper, to remove the adhering alcohol), and transferred to a watch-glass containing oil of cloves. By this means it becomes, in a few seconds, quite transparent. If it is coloured, the colour becomes more intense; if it is unstained, it becomes almost invisible. From the oil of cloves it is transferred by the same means to a drop of Dammar varnish, previously placed in the centre of an object-glass.*

If excessively delicate and thin sections are to be mounted, such, *e.g.*, as sections of the retina, or of any thin membrane, it is not possible, without risk, to transfer them from one liquid to another. In this case it is, therefore, necessary to swim the section directly from the knife on to the object-glass, in which position they must be treated with the several liquids to be employed; and each liquid must be allowed to fall on to the section, and, after producing its effect, removed by inclining the glass, care being taken not to allow the object to float away at the same time. All delicate sections must be protected by the interposition, between the object and cover-glass, of a square of silver paper, with a window cut in it somewhat smaller than the latter.

Methods of Preserving Preparations permanently.—Preparations which are to be preserved must be mounted permanently. Those which are in liquids, such as glycerin, acetate of potash, bichromate of potash, &c., must be surrounded with cement, in order to fix the cover-glass. For those which are in glycerin jelly, Canada balsam (neither of which, however, are to be recommended), or Dammar varnish, that is not necessary. Various kinds of varnish are used for the purpose, such as Frankfort lac, asphalt, &c. I use always Dammar varnish. A streak of the varnish is placed on the edge of the cover-glass and carried all round it, with the aid of a glass rod drawn to a point, or a brush, care being taken that it extends only a very little over the cover-glass. Before applying the varnish, the excess of liquid must be carefully removed with blotting-paper from the edge of the cover-glass. I dispense with the instrument frequently used for mounting, for the following reasons:—If the cover-glass is already fixed, as, *e.g.*, in Canada balsam or Dammar preparations, any additional mounting is unnecessary. If it is not fixed, *i.e.*, when the medium in which the preparation is contained is liquid, there is much greater risk of displacement with the machine than without it. It should always be borne in mind that the preservation of the preparation is of more importance than the outside setting. The other kinds of varnish may be used instead of labels, for writing on the glass the name of the preparation. If it is desired to preserve a preparation already covered in water and solution of osmic acid, or bichromate of potash, &c., without removing the cover, so as to avoid risk of displacement, the best way is to irrigate it with glycerin or acetate of potash, until the one liquid is replaced by the other. The excess of liquid must then be removed with blotting-paper, and the cover-glass surrounded with Dammar varnish. If, by inadvertence, the upper surface of that part of the cover-glass which is above the preparation has been smeared with glycerin or Dammar varnish, and it is desired not to remount it, the only way is to wait until the setting is dry. The spot can then be removed with a camel-hair pencil soaked in water if it be glycerin, or in turpentine and afterwards in alcohol if it be Dammar.

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* The lifter or spoon may be made by flattening the end of a copper or german-silver wire, and bending it at right angles. It is desirable to place the object-glass on a white ground if the object is stained, or on a black ground if it is unstained, in order that the folds, if present, may be seen and removed. If several sections are to be placed under one cover-glass, each section may be pressed gently down on the surface of the glass before covering; the sections then adhere to the glass sufficiently to keep in their places.

CHAPTER VII.

VASCULAR SYSTEM.

Section I.—Methods of Injection.

Before describing the structure of the blood-vessels and lymphatics, an account will be given of the methods of injecting. The processes of injection may be divided according as they are used during life or after death.

Methods of Injecting during Life.—The method of injecting the vessels of an animal during life has, hitherto, not been much employed. It may be practised either for the purpose merely of introducing into the circulation any suitable liquid containing colouring matters, or other substances in solution or suspension, or with a view to emptying the vessels of their contents and substituting another liquid. For example, we have already seen that insoluble colouring matters are introduced in order to feed the colourless blood corpuscles and those of the connective tissue; and we shall subsequently see that by the injection of coloured solutions, a "natural injection," produced by excretion of the ducts of certain glandular organs, may be obtained. (*See* Chapter X.) The most important insoluble colouring matters are vermilion, carmine, and anilin, which are used suspended in salt solution, as described in Chapter I. p. 11. Insoluble Prussian blue, as precipitated by the gradual addition of alcohol to the solution, can also be used in the same way.

The methods are as follow:—

Injection of the Frog during Life.—In a large frog, secured on its back, the abdominal vein is carefully exposed under a dissecting lens, in its course up the middle line of the anterior wall of the belly. A ligature is passed round the distal end of the prepared part and tightened. A small clip is then placed on the proximal end, and a ligature passed under the vein between the two, which is looped, but not tightened. The vein having then been opened just beyond the loop, with a pair of sharp scissors, a fine glass cannula is introduced in the direction of the circulation. The loop is then tightened round the cannula and knotted. The cannula must now be filled, with the aid of a capillary pipette, with salt solution, and connected by a bit of india-rubber tubing with a brass syringe, in doing which great care must be taken not to tear the cannula out of the vein. If it is desired to continue the injection for some time, it is better to employ the pressure of a column of liquid, for which purpose the following arrangement must be used:—A moderate-sized flask, containing the injection liquid, is supported on a retort-holder at a height of about two or three feet above the table. The flask is fitted with a cork, in which two tubes are fixed, the one being straight for the admission of air, the other bent so as to form a syphon, the short leg of which dips under the level of the liquid. To the other end an india-rubber tube, furnished with a screw-clamp, is fitted, long enough to reach the cannula. A current is now produced along the tube by suction, which can be regulated by the clamp so as to allow the liquid to flow in a rapid succession of drops. The tube is then momentarily closed by a second clip, and connected with the cannula. The clip on the tube is now opened and that on the vein removed. As soon as the injection is finished the vein is ligatured on the proximal side of the cannula, which is then withdrawn. In long injections, it is of course necessary to open the peripheral end of the vein. If it is desired to estimate the quantity of liquid injected, a cylindrical bottle is substituted for the flask, which must be previously graduated. When the object in view is to replace the blood completely with salt solution (with or without colouring matter), it is better to introduce the cannula into the *bulbus arteriosus*.

Injection of small Mammalian Animals during Life.—The animal is secured with the aid of Czermak's holder. (*See* Chapter XVI.) The external jugular is then exposed by a sufficient incision, and cleared of the surrounding tissue with the aid of dissecting forceps. The vessel having been ligatured at the distal end of the prepared part, and a clip placed on it at the central end, the vein is opened by a small incision, and a proper cannula inserted and secured with a ligature. The cannula is then filled with salt solution with the aid of a capillary pipette, and connected either with the syringe or the tube of the syphon previously described. Finally, the clip is opened, and the liquid allowed gradually to enter the vein. As soon as the injection is completed, the clip is immediately closed. Before the cannula is removed, the vein is of course ligatured. If the quantity to be injected is small, it is simpler to use a small subcutaneous syringe, in which case all that is necessary is to compress the vein immediately above the clavicle, and to pierce it, when distended, with the point of the cannula. The pressure having been discontinued, the liquid is at once injected. The aperture must be seized by means of clip-forceps as the cannula is withdrawn, so as to prevent bleeding.

Injection after Death.—The materials used for this purpose are Prussian blue, carmine, and nitrate of silver.

Prussian blue, like carmine, can be injected either in solution or suspension in water, or in solution in gelatin. Silver is mostly used in solution in water. Soluble Prussian blue, which is more used for injection than any other colouring matter, is prepared according to the method of Brücke. 217 grammes of ferro-cyanide of potassium are dissolved in a litre of water in a large flask (Solution A). In another flask a solution (B) of chloride of iron is prepared, containing one part of the salt in ten parts of water. A third solution (C) is prepared of sulphate of soda, which must be saturated. Equal parts of the solutions A and B are mixed, each with twice its bulk of C. The chloride of iron mixture is then poured slowly into the mixture containing the yellow prussiate, care being taken to stir constantly during the addition. The precipitate having been allowed to settle, the greenish supernatant liquid is poured away and the residue thrown into a flannel strainer. The blue liquid which passes through is returned to the strainer until it becomes transparent. Thereupon what remains on the filter is washed with water until what passes through is of an intense blue colour. The filter is allowed to drain completely, and then placed between shreds of blotting paper, and left to dry gradually in a sufficiently cool place. It is then broken up into small fragments and kept in a glass bottle. The blue material so prepared is perfectly and readily soluble in water.

A two per cent. solution of this material may be used either at the ordinary temperature or at the temperature of the body. It can be injected with great facility. When it is used with gelatin, the mass is prepared by adding five parts of the filtered solution above mentioned to one hundred parts of a solution of gelatin, containing one part of gelatin to eight of water. The gelatin is first dissolved in the water over a water-bath in a porcelain dish; the hot solution is then filtered through flannel or fine calico, it is replaced on the water-bath, and the blue liquid is gradually added to it with constant agitation.

[There are some other blue liquids of the same kind in use: "*Beale's Prussian blue fluid*" is prepared as follows:—Take one ounce of common glycerin, one ounce of spirits of wine, twelve grains of ferro-cyanide of potassium, one drachm of tincture or solution of perchloride of iron, and four ounces of water. The ferro-cyanide is dissolved in half an ounce each of water and glycerin, and the iron mixed with similar quantities of both ingredients. The chloride of iron mixture is thereupon added gradually to the ferro-cyanide, with constant agitation. Finally, the spirits of wine and the remainder of the water are added gradually.

"*Turnbull's Blue.*"—Ten grains of protosulphate of iron are dissolved in an ounce of glycerin diluted with a little water. Thirty-two grains of ferro-cyanide of potassium are dissolved in the same quantity. The iron is then added to the red prussiate with constant agitation. Beale modifies this formula by substituting five grains of sulphate of iron and ten of the red prussiate for the quantities above stated, and adding to the mixture an ounce of water and a drachm of alcohol.]

Carmine.—A mass which is fluid at ordinary temperatures is prepared, according to Beale, as follows:—Take five grains of carmine, half an ounce of glycerin containing eight or ten drops of acetic acid, one ounce of pure glycerin, two drachms of alcohol and six drachms of water. The carmine is first mixed with a little water containing about five drops of ammonia. Half an ounce of pure glycerin having been added to this liquid, it is shaken in a flask, and then gradually poured into the acidulated glycerin, with constant agitation. If the mixture is not distinctly acid, a trace of acetic acid is added to the remaining half ounce of glycerin, which with the alcohol and water is then gradually added to the rest. It is necessary to prepare this mixture each time that it is used. The alcohol may be omitted altogether without detriment. Carmine is usually employed in solution of gelatin. The following liquids are to be recommended:—

Gerlach's Carmine Mass.—Sixty-nine grains of carmine are dissolved in seventy grains of water with eight drops of liquor ammoniæ. The solution, having been exposed to the air for several days, is mixed with a solution of one and-a-half drachm of gelatin in one and-three-quarter drachm of water. A few drops of acetic acid are added to the warm mixture. *Dr. Carter's Carmine Mass.*—Take sixty grains of carmine, 120 grains of liquor ammoniæ, eighty-six minims of glacial acetic acid, two ounces of solution of gelatin, containing one part in six, one and-a-half ounce of water. The carmine is dissolved in the ammonia and water and filtered. The filtrate is added to one and-a-half ounce of solution of gelatin. The other half ounce is mixed with the acetic acid, and added *guttatim* to the rest, with constant agitation.

I found this mass answer extremely well with the following modification:—Four grammes of carmine having been suspended in a few drops of water, eight cubic centimeters of liquor ammoniæ and forty-eight cubic centimeters of water are added. As soon as the carmine is dissolved, the liquid is filtered—a process which requires several hours. A gelatin solution, containing one part in eight of gelatin, is next prepared and filtered through fine calico. The carmine solution is added gradually to two ounces of the filtrate, which is kept warm over the water-bath. Forty or fifty minims of glacial acetic acid are then added to another half ounce of warm gelatin solution, which is mixed gradually with the rest, with constant agitation. Before the whole of the acid gelatin is added, the mixture changes its colour from bright red to dirty red. By the addition of the last drops, the mass acquires the slight acid reaction which is necessary to render it indiffusible in the tissues.

Silver Solution.—The solution of silver used for injection contains one-quarter or half per cent. of the salt.

Apparatus and Instruments.—*Syringes* of the ordinary form answer well. They may be made of brass or german silver. They are, however, now used only for special purposes, *e.g.*, for the injection of very small organs, and are open to the objection that much practice is required in order to regulate the pressure in such a way as to ensure success; deficient pressure rendering the injection imperfect, too much producing extravasation. In general, and indeed in all cases in which it is desirable that the pressure should be constant throughout, the apparatus to be hereafter described must be used. *Cannulas.*—When the syringe is used, it is better to employ metal cannulas than glass ones. The former consist of three parts (Fig. 17), viz.—a collar, with two cross arms, and a tubular beak. The beak is bevelled at the end, and is grooved at a short distance from the bevelling. The dimensions of the whole are accurately shown in the drawing. The point must be carefully rounded. The nozzle of the syringe is plugged into the collar, and is fitted with a stop-cock, in order to prevent the mass from returning after the injection is completed. This object can also be answered by a ligature, but in many cases this would be difficult from want of space. Three or four such cannulas with beaks of different calibres are necessary. Glass cannulas should be made of the following form:—A tube is drawn out in such a manner that it tapers to a degree which varies according to the size of the vessel into which it is intended to be introduced. The end must be truncated and smooth, and must have a constriction at a distance of about three millimeters. The large end should also be a little drawn out, so that an india-rubber tube can be easily slipped over it, and secured.

The various forms of apparatus for injection all depend on the principle that the pressure which is required for injecting is produced by the influx of water or mercury into a closed vessel. The mechanical arrangements employed for this purpose are as follow:—A bottle containing water is suspended by a pulley, so that it can be raised to any required height. From a tubulature near the bottom, a flexible tube issues which reaches to the table, and is connected with a glass tube, which is fitted by a cork into one of the tubulatures of a large Woolff's bottle, the bottom of which it almost touches. In the other neck of the bottle a cork is also fitted, which contains a short glass tube bent at the top; this is connected by a flexible tube with the stem of a T shaped tube, one branch of which leads to a manometer, the other to a second smaller Woolff's bottle, in which the injection mass is contained. The long flexible tube which leads from the suspended bottle must be furnished with a clamp, and another is required on the tube which connects the T with the injection flask.—Another arrangement consists of a large flask holding several gallons, in the mouth of which a large india-rubber stopper can be fitted. At the bottom there is a side tubulature (for discharging the water when necessary), into which a second stopper must be fitted. The stopper contains a strong glass tube, having a bit of india-rubber tube fitted to it, guarded by a strong clamp. In the large stopper are two glass tubes, one of which is short, not extending beyond the neck, and bent at the top; it is connected with a T tube, which corresponds to the one employed in the apparatus first described. The second tube is of the same form as the first, and communicates with a supply tap.—In other forms of apparatus mercury is used. The apparatus may then consist merely in a single Woolff's bottle, into one of the necks of which a rose funnel is fitted, reaching to the bottom. The other neck contains a short bent glass tube, which communicates with the T tube as before.* In all forms of apparatus for injection, it is necessary to take the greatest care to make all the junctions absolutely air-tight.

The injection mass is always contained as above described in a Woolff's bottle, which should be previously graduated, so that the operator may know as he proceeds how much has been injected. One of the necks of the bottle is in communication with the T tube, by means of a short glass tube fitted with a caoutchouc connector, which does not reach below the vulcanite stopper in which it is fixed. In the other, a long tube is contained, the end of which reaches to the bottom of the bottle, while the top communicates with the cannula. If a metal cannula is used, the india-rubber tube is fitted on to the stop-cock. If the cannula is of glass, it is guarded by a screw-clamp.

When the organ or animal to be injected is small, it answers well to use the syringe as a compression air-pump, by connecting it with the short tube of the Woolff's bottle. The superiority of this method over the direct use of the syringe is obvious. The inequalities of the pressure, which are its chief disadvantage, are annulled by the elasticity of the air contained in the bottle, which serves as a kind of cushion. While the operator fixes his attention on the cannula, an assistant gradually injects air into the bottle until the contents of the syringe are discharged. The tube must then be closed with a screw-clamp, and the operation, if necessary, repeated.

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* Of the more complicated forms of mercurial apparatus, that devised by Hering (which is to be had of Heinitz, instrument-maker in Vienna) is undoubtedly the best, and answers all requirements. A description of it will be found in the Wiener Sitzungsberichte.

When warm masses are used, it is commonly necessary to place the injection-bottle in a water-bath, kept warm by a spirit lamp. It is also desirable to keep the object warm, for which purpose it is placed on a plate of glass over a water-bath; or (as in Ludwig's arrangement) a warm chamber of metal supported on a tripod is used, which is large enough to hold both the animal and the bottle containing the injection. It is furnished with a cover and air opening for the admission of the compressed air.

In order to illustrate the method more completely, I will describe three injections. In the first of these examples the syringe is used in the ordinary way; in the second it serves as a pump for the injection of air into the Woolff's bottle containing the mass; in the third, the apparatus is used. Suppose that it is desired to inject the kidneys of a small mammal with cold two per cent. solution of Prussian blue. The animal having been just killed by bleeding, the abdomen is opened and the whole mass of intestines pushed aside to the right. The left renal artery is then separated from surrounding parts with the aid of two pairs of ordinary forceps without any cutting instrument. A silk ligature is placed round the artery, and looped near to the point at which it enters the kidney. The vein is next prepared in the same way, and a ligature placed round it close to its junction with the vena cava. By drawing on the renal vein, it is easy to make a valvular opening with fine scissors. The artery is similarly opened short of the loop, and the metal cannula with its stop-cock introduced, the edge of the incision being held aside with the forceps. In making the opening and inserting the cannula, the greatest care must be taken to avoid rupturing the artery or cutting it through with the scissors. The moment that the cannula is in the artery, the loop must be tightened round the groove. The cannula and nozzle are then filled with half per cent. salt solution with the aid of a capillary tube: the syringe is charged with the liquid and connected with the nozzle. In injecting, the piston must be slowly pushed forwards. As soon as the organ becomes blue, and the liquid appears to pass unmixed from the opening in the vein, I stop, and then direct my assistant to close the vein with a clip, or to tighten a loop previously placed round the vessel for this purpose. This done, I make one push more with the piston, turn the stop-cock of the nozzle, and take away the syringe.

I will next describe the injection of a whole animal, such as a rat or a small rabbit, with carmine gelatin mass. The animal is killed by inhalation of chloroform. A window is then cut out in the left wall of the chest, just large enough to expose the heart and the roots of the great vessels, taking care not to carry these incisions so near the middle line as to endanger the internal mammary artery. A fold of pericardium having been taken up with the forceps and divided, the apex of the heart is raised out of the thorax and pierced with a threaded needle through both ventricles. By the thread which has been brought through, the apex is then drawn downwards by an assistant, while the root of the aorta is cleared with the aid of two pairs of dissecting forceps. A ligature is then passed round it close to its origin, and looped. Thereupon the wall of the left ventricle is opened near its base, and as soon as blood has ceased to flow, the cannula is passed into the aorta, to such a distance that its neck can be grasped by the ligature, which is then tightened. The blood in the cannula is then removed with a capillary pipette, and filled with saline solution with another pipette, and an opening is made in the right ventricle. Up to this time the animal has been allowed to remain on a plate. The plate is now placed on a support, at a level which nearly corresponds with that of the Woolff's bottle, in which the mass is contained, which is kept warm by immersion in a water-bath, heated by a spirit lamp. The nozzle having been connected with the discharge tube of the flask by an india-rubber tube, and the syringe (the piston of which has been drawn up) with the other opening in the Woolff's bottle, an assistant injects a little air so as to fill the discharge tube up to the orifice of the nozzle. The stop-cock is then closed, and the point of the nozzle inserted in the cannula. The stop-cock having been reopened, the assistant pushes on the piston. As soon as the syringe is emptied, the screw-clamp between it and the injection bottle is tightened. Air is again injected, if necessary, in the same manner. If, however, a full-sized syringe is used, it is seldom necessary to repeat the process. When the vessels are sufficiently full, the heart is seized with strong clip-forceps, as near the base as possible, care being taken not to include the cannula. The stop-cock is then closed.

As a third example may be taken the injection of the abdominal organs of a rabbit. The animal is decapitated. The whole of the left wall of the thorax is removed from the flanks forwards as far down as the costal origin of the anterior half of the diaphragm. The left lung and the heart having been drawn aside to the left, the thoracic aorta is prepared with two pairs of dissecting forceps as far down as possible. A ligature having been passed round the vessel and looped, and the vessel slit open, the cannula is introduced and the ligature tightened. The cannula having been then cleared of blood and filled with saline solution, the plate on which the animal lies is put into the warm chamber which contains the injection bottle. This bottle, which is charged with the warm Prussian blue mass, is connected with the pressure bottle, the manometer of which indicates a pressure of 60 to 120 millimeters. It is, however, not in communication with it, for the connecting tube is closed by a clamp. This clamp is then slightly opened for a moment, so as to fill the discharge tube to the orifice, and immediately closed, the stop-cock being shut at the same time. The nozzle having been inserted into the cannula, the stop-cock and clamp are simultaneously opened. The cover of the chamber is put on and the injection allowed to proceed, all that is required being to maintain the pressure in the apparatus as nearly constant as possible. When the injection is complete, a clip is placed on the vena cava, near its mouth, and the stop-cock shut. [The special methods to be used for the injection of particular organs, and the methods of double injection, will be given under the proper heads.]

Injection with Solution of Nitrate of Silver.—It is preferable for this purpose to work with the apparatus, as it is necessary to employ a considerable pressure. As soon as the injection is completed, it must be replaced by water. This is effected by substituting a flask containing water for that used for the nitrate of silver solution. The vessels must be thoroughly streamed with water, otherwise the endothelial markings are concealed by the quantity of precipitate which is formed.

Treatment of Injected Tissues.—Organs injected with coloured masses must be suspended in ordinary alcohol in a beaker. If a whole animal has been injected, the body must be left to cool for half an hour or more. It must then be transferred to a large vessel containing common alcohol, to which a few drops of glacial acetic acid have been added. It is a good plan to transfer animals which have been injected with gelatin masses to ice-cold alcohol, immediately after the completion of the injection; great care being taken in this, as in every other case, to secure the artery and vein so as to avoid all risk of escape of the mass.

SECTION II.—STRUCTURE OF THE BLOOD-VESSELS.

Endothelium.—The simplest method of demonstration is to colour the internal surface with silver. If the vessels are of large size, they are prepared as follows:—A portion of the vessel taken from the freshly killed animal is washed with diluted serum and then dipped for a few minutes in half per cent. solution of silver. Its internal surface is then exposed to light until it acquires a brownish-yellow colour. If the muscular wall is thick, the intima must be separated by the method previously described (Chapter II. p. 34) and covered in glycerin, with its endothelial surface upwards. If the vessel is thin-walled, *e.g.*, the vena cava of a small animal, it can be covered without any preparation. For the endothelium of capillaries in the kidney or bladder, or in the serous membranes, the best results are obtained by injection of the solution of nitrate of silver. In the serous membranes, however, *e.g.*, in the mesentery, good preparations can be obtained by first pencilling one or both surfaces with fresh serum *in situ* (the animal having been bled to death), and then cutting out the pencilled part and colouring in silver in the usual way.

The endothelium of the large arteries consists of long narrow spindle-shaped plates. The nucleus is oblong, and usually in the middle of each plate. The interstitial lines are very slightly sinuous. The endothelial elements of the veins are relatively broader. If the staining is intense, the cell is filled with brown precipitate, the nucleus remaining clear. The capillary vessels appear, when coloured with silver, to consist merely of oblong plates, the interstitial lines of which are commonly more or less sinuous. The oblong regular nuclei of the walls of the capillaries seen in profile are those of the endothelial elements. It is easy to colour the nuclei by carmine, in which case an acid solution must be used, *i.e.*, an ammoniacal solution to which a sufficient quantity of acetic acid has been added to render it distinctly acid. Larger vessels must be immersed in the solution, but for capillaries it is enough to immerse the membrane in which they are contained. Ten minutes immersion is sufficient for the purpose: the preparation must then be washed in water and prepared in glycerin. In preparations of mesentery of the frog or of a small mammalian animal in bichromate of potash, the nuclei may be readily recognized, not only in profile, but on the surface of the small vessels.

The Intima.—The anatomical relations of the intima, *i.e.*, of the internal longitudinal fibres, and the elastic membrane, may be studied either in sections or in the fresh state. In large arteries, the best method is to immerse the vessel in one per cent. solution of bichromate for several days. The intima is then peeled off in thin strips, which are teased in the same liquid and covered with glycerin. This is the only way of showing the elastic network or the fenestrated membrane which exists in certain arteries. In vessels of macroscopical dimensions, *e.g.*, in large arteries of the mesentery, the intima is seen in profile both in fresh preparations and after treatment with bichromate of potash, as a doubly-contoured, sharply-defined membrane; the surface view showing traces of longitudinal fibres. In cross sections of smaller arteries, the intima is seen as a wavy hyaline membrane, differing in thickness according to the size of the artery. The intima of large veins differs only in its thickness from that of the arteries.

Muscular Coat.—The muscular coat differs in its characters in different parts of the vascular system. In the arteries, the muscular elements form layers which are connected together by the elastic lamellæ interposed between them, the two together constituting the *tunica media*. In many arteries there are also bundles of muscular fibres in the intima, and in others in the adventitia. In the minute arteries they form circular layers, the number of which varies according to the size of the vessel. In such arteries the media is made up almost entirely of muscular fibres.

The muscular fibres of large arteries may be studied either by teasing preparations of vessels steeped in bichromate of potash, or in sections hardened in chromic acid. The muscle-cells of large arteries appear, when isolated, to be broader, relatively, than ordinary muscular elements, and are often split at their ends into processes. The oblong nuclei are more or less staff-shaped.—If a portion of fresh bladder of the frog is treated with acetic acid in the manner already recommended in the chapter on unstriped muscular fibres, or a portion of mesentery of a frog or mammal with bichromate of potash or acetic acid, the muscle-cells can be distinguished as transversely arranged short spindles, enclosing long distinctly granular staff-shaped nuclei, which are arranged in rows alternating with each other. In optical longitudinal sections of minute arteries, such as occur very frequently in sections of hardened tissues, the elements of the media exhibit the same appearances as in cross sections of involuntary muscle in general; as, however, the muscle-cells are shorter, and their nuclei longer, most cross sections exhibit a nucleus in almost every element. In minute veins, muscular elements are seen which have a longitudinal direction, but do not form a continuous layer.*

The intima and adventitia of the blood-vessels contain numerous branched cells. To demonstrate them, sections must be made of blood-vessels and treated with gold. They may be also shown in preparations made by the silver method. By this method a rich network of lymphatics may be demonstrated in the adventitia of the aorta of small animals.

Nerves.—The rich plexus of non-medullated nerve fibres which exists in the adventitia of large blood-vessels, can be studied in the mesentery of the frog, which for this purpose must be prepared in the manner directed in Chapter V. for the demonstration of the nerves of the mesentery. In the same organ it can be shown that the capillaries are also surrounded by non-medullated nerve fibres. In the nictitating membrane and tongue of the frog, the plexuses which surround the capillaries may be seen to give out fibrils which enter the walls of the vessels themselves. For this purpose the tongue of the frog must be coloured in a half per cent. solution of chloride of gold, and used, after hardening in alcohol, for the preparation of sections. (*See* Chapter XII.)

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* For the special arrangements of the muscular fibres in particular arteries, the reader is referred to larger treatises on general anatomy.

The development of blood-vessels will be given in the chapter on Embryology.

SECTION III.—MICROSCOPICAL STUDY OF THE CIRCULATION.

Study of the Circulation in Cold-blooded Animals.—The parts which may be used for this purpose are (1) the web of the frog's foot, (2) the mesentery of the frog or toad, (3) the tongue of the same animal, (4) the tadpole.

Web of the Frog.—If the animal is not curarized, the arrangement must be employed which was described in Chapter III. It is, however, better to employ curare, as described in Chapter XVII. The animal is laid on an oblong plate of glass, on which a cork disk is fixed with sealing-wax, which should be three-tenths of an inch thick, and an inch and a quarter wide. The disk must have a hole in the middle, which should be about three-quarters of an inch wide. At the edges of this aperture pins are stuck, to which ligatures attached to the toes may be secured.

Mesentery.—The preparation of the mesentery is not so simple. A snip is made in the right side of the belly, parallel with the middle line. Before dividing the skin further, it is raised to ascertain where there are no large veins; the incision is then continued upwards and downwards, in such directions as to avoid bleeding. If, notwithstanding, a vein is divided, the bleeding must be restrained by seizing the end of the incision with the clip-forceps. The traces of blood having been removed with filter paper, the muscles are divided in the same vertical line. This having been done, the intestine and mesentery are drawn out carefully, and laid on the anterior surface of the belly. The next step is to place the animal on *a* in Fig. 19. (For this, however, a simple glass plate of similar size may be substituted, at the edge of which a cork is fixed, which should have an aperture corresponding to *c*, covered with a round cover-glass.) The frog having been pushed up against *d*, the intestine can be easily turned over on to *b*. The intestine then lies in the trough *c*, while the mesentery rests on the glass plate *b*. So much of the intestine as does not occupy the trough must be replaced. If the observation is prolonged (as in researches on inflammation), it is well to place in the trough, outside of the intestine, a layer of filter paper, on which half per cent. solution of salt is dropped from time to time. It is sometimes useful, when high powers are to be employed, to cover the mesentery with thin glass. If the cork is used, it is necessary to fix the intestine at two or three points with small pins.

Tongue.—The animal must be curarized as before. A plate of glass, like that used for the web, is employed, with this difference, that the cork, instead of having a round aperture, is cut into the form of a horseshoe, the convexity of which is towards the edge of the plate. If it is intended to study the circulation on the lower surface of the tongue, the animal is placed on its belly. If the papillary surface is to be examined, it must be on its back. In either case, the tongue must be drawn out by the cornua, around each of which a thread must be secured. With the aid of these threads, the organ is drawn as forward as possible without affecting the circulation, and secured to pins which are stuck horizontally into the edge of the cork at each corner. It is sometimes necessary to extend the organ further by means of pins stuck in the cork at the sides.

Tail of the Tadpole.—The tail of the tadpole affords a most instructive object. The animal is curarized by placing it in a drop or two of solution in a watch-glass. As soon as it is motionless, it is transferred to an object-glass and examined. The description of the phenomena of circulation as seen in the batrachians, and of the methods employed for their investigation in mammalia, will be found in Chapter XVII.

Observation of the Emigration of Coloured and Colourless Blood Corpuscles.—In the tadpole, emigration, particularly of the coloured corpuscles, may be witnessed in various parts of the tail, if the observation is continued for a short time.* If the mesentery of a frog is exposed to the air, or treated with any irritant, the emigration of colourless corpuscles can be seen with the greatest ease, provided that the observation is made with sufficient care. A small vein must be sought out with a low power, and a point selected in its course at which one or more colourless corpuscles have attached themselves to the walls. These must then be watched continuously under a higher power.

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* In the frog an abundant emigration of coloured corpuscles takes place after the injection of salt solution (two to six per cent.).

CHAPTER VIII.

LYMPHATIC SYSTEM.

SECTION I.—LYMPHATIC VESSELS.

THE lymphatic vessels may be studied either by colouring with nitrate of silver or by injection. As those of the serous membranes are most readily demonstrated, it will be convenient to refer to them first.

Lymphatics of the Centrum Tendineum of the Diaphragm.—The pleural cavity of a rabbit or guineapig, which has just been killed, is exposed by removing the sternum, care being taken to avoid opening any large blood-vessels. The pleura having then been divided along the edge of the costal part of the diaphragm, the *cava ascendens* is ligatured close to the atrium, and divided between the ligature and the heart. The heart and lungs are then removed from the thoracic cavity. The pleural side of the *centrum tendineum* is then carefully brushed with a camel-hair pencil, moistened with serum, after which a small quantity of half per cent. solution of nitrate of silver is poured on the diaphragm, while the animal is held vertically, with its head uppermost. After five minutes or so, the silver solution is poured away and replaced by water, which should be changed several times. The *centrum tendineum* may then be cut out, and prepared in glycerin. According to another plan, the diaphragm is cut out immediately after it has been brushed, and immersed in solution of nitrate of silver. With this view the abdominal cavity is opened; the *ligamentum suspensorium* is divided, and a ligature placed round the vena portæ. This vein having been divided, the whole diaphragm is cut out with the liver. In such a preparation clear channels are seen in the yellowish-brown ground-substance, which are of various size, and of two kinds, and exhibit endothelial markings. In the one kind—viz., in the larger vessels—the endothelial elements are spindle-shaped; in the other—*i.e.*, the capillaries—they are more or less sinuous. The walls of all these vessels consist exclusively of endothelium.

Before describing the arrangement of the lymphatics in the *cen-*

trum tendineum, it is desirable to give an account of the structure of that organ. It consists of three parts, viz., pleura, peritonæum, and tendon; each serous membrane being made up of *endothelium* and *membrana propria.* The tendon consists of two layers, of which the one that is next the peritonæum is formed of bundles of fibres, which radiate from the centre outwards; the upper layer of bundles arranged circularly. The bundles of each are separated from their neighbours by splits or channels, of which there are two sets; those between the abdominal layers being designated the superficial, those of the pleural, the deep interfascicular channels of the *centrum tendineum.* The *membrana propria* of the peritonæum, where it stretches over the superficial channels, possesses a special fenestrated structure (found also in one or two situations elsewhere). Between the propria of the pleural side and the tendons, large lymphatic vessels exist which form numerous ramifications, and communicate with a network of capillaries. All of the larger vessels are provided with valves, with their corresponding dilatations. The capillaries may be distinguished into those which lie in the pleural propria, and have a more or less winding course, and those which are straight and lie further from the pleural surface. The former have saccular dilatations, which are called lymphatie sinuses. The straight vessels are contained in the channels already described, and may, therefore, be designated lymphatics of the interfascicular channels. They may be further distinguished, according as they are contained in the peritonæal or pleural layer, into superficial and deep. There are many channels of both layers which do not contain them. The two sets of vessels are in communication with each other. The superficial interfascicular lymphatics pass, in the neighbourhood of the great vessels which perforate the *centrum tendineum,* into winding lymphatic capillaries with saccular dilatations, which are situated on the abdominal surface of the tendon, where they form a network. On the other hand, the interfascicular lymphatics freely communicate with the peritonæal cavity by means of vertical channels, which, although they for the most part extend only to the radiating lymphatics of the superficial layer, can also, in many instances, be seen to pass directly to those contained in the deeper, *i.e.,* the circular channels. By these canals the endothelium of the lymphatics is continuous with that of the peritonæum. The endothelial elements which guard the orifices of each vertical canal (the stoma) have the characters of young cells, and differ from those which adjoin them in being more granular, smaller, and polyhedric. It has been already indicated, in Chapter II., that the endothelium which covers the channels consists of smaller and apparently younger elements than those of the general surface. These characters are much more marked in the cells which surround and form the stomata. In diaphragms which have been stained without brushing, they cannot be made out. It is true that, among the small mosaic of certain channels, there are dark or clear spots which have been described by authors as stomata, with which, however, their relation is very doubtful.

Method of Demonstrating the Stomata.—To demonstrate them, the abdominal cavity of a rabbit just killed must be opened, a ligature passed round the cardia, and another round the bunch of vessels leading to the porta. This done, the abdominal viscera, excepting the liver, may be cut away and removed; great care being taken not to draw upon the diaphragm in any part of the operation. The liver being then held aside, water is poured over the abdominal surface of the diaphragm. After a few seconds, silver solution is poured once or twice over it in the same way, and the whole left to itself for a few minutes. It is then again washed with water, after which it may be cut out and subjected to microscopical examination. In preparations so obtained rows of stomata may be seen, both over the superficial interfascicular lymphatics, and occasionally in situations which correspond to the circular ones; which exhibit, in all respects, the same anatomical characters as those of the *septum cisternæ magnæ* in the frog. Each canal leading from a stoma to a subjacent lymphatic is seen to be lined by small granular cells of the same character as those already described as guarding the orifice. They are particularly distinct where the canal opens into the lymphatic, especially in those canals which are in communication with the lymphatics of the deeper, *i.e.*, the pleural layer. The lymphatic system of the diaphragm is divisible by the middle line into two similar halves. Each half may be again divided, according to the direction in which the lymph flows, into two parts—an anterior and a posterior. The anterior system is made up of the large lymphatic vessels to be found on the pleural side, all of which converge towards the sternum, discharging themselves into a single large lymphatic trunk, which stretches in the form of an arch along the outer edge of the sternum, accompanying the internal mammary artery and vein.

Each of the trunks as it ascends divides into a plexus of smaller vessels, by which the lymph is conveyed to the sternal glands. These lymph vessels receive their tributaries from the external border of the anterior half of the centrum, and from the anterior third of the external border of the posterior half. The lymphatic vessels of the remainder of the diaphragm belong to the posterior system, which opens on either side by a short, wide lymphatic trunk, which joins the thoracic duct just after the latter has entered the thoracic cavity. The lymphatic interfascicular channels are all to be regarded as tubes of communication between the two systems. From these facts it may be understood why the posterior half of the diaphragm is more readily filled from the peritonæum than the anterior.

Demonstration of the Lymphatic System of the Diaphragm by Injection.—In a large or middle-sized rabbit, which has been kept from sixteen to twenty hours without food, ten cubic centimeters of a warm, five per cent. solution of Prussian blue are injected into the abdominal cavity through a small cannula, with the aid of a glass tube drawn out at one end. The liquid is allowed to flow in of itself. After three hours and a half, the animal is bled to death by opening the carotid artery, or killed by strangling. As soon as the body is cool, the pleural cavity is opened, the *cava ascendens* is ligatured just before it enters the heart, while a second ligature is tightened round the aorta, œsophagus, thoracic duct, and *vena azygos*. The vessels having been divided above the ligatures, the whole of the thoracic viscera are removed. With a lens the arrangement of the vessels above described may now be made out, without removing the diaphragm. To obtain permanent preparations, the peritonæal cavity must be opened, and the suspensory ligament divided as before directed, the animal being placed aslant. The *vena cava* and the cardia having next been divided between the liver and the diaphragm, the serous ligaments which connect the left lobe of the liver, the stomach, and the spleen with the diaphragm, are severed, so that these organs are completely detached. Thereupon the abdominal surface of the *centrum tendineum* is brushed with a camel-hair pencil moistened with warm water, after which the ring of bone, cartilages, and soft parts, to which the diaphragm is attached all round, is separated from the rest of the body, immersed for a few minutes in silver, and washed in water. Those parts which are intended for microscopical examination can then be cut out and covered in glycerin. Anilin and milk may be used in the same manner as Prussian blue, but do not yield such certain results.

Another method of injecting the lymphatics of the diaphragm may be mentioned, which is, however, not so successful. The liquid employed consists either of one or two per cent. solution of Prussian blue, in which a partial fine precipitation has been determined by the addition of a small quantity of alcohol, or of anilin with milk. A rabbit is bled to death by opening the crural artery. A bent tube is then secured in the trachea, which is connected with the apparatus for artificial respiration. The abdominal cavity is then opened and the suspensory ligament divided, as well as the fold of serous membrane which connects the left lobe of the liver with the diaphragm. The cardia having been exposed and tied, and a ligature passed round the vessels contained in the *omentum minus* and the *vena cava* below the liver, the organs are cut away below the ligatures, so that the diaphragm is covered only by the liver. The lumbar part of the spinal column is then severed, and the division completed by continuing the incision forwards on either side to the middle line. Threads are then attached to the cut edges, by which the upper part of the body is suspended, head downwards, to a ring of iron. The whole operation can be completed in from three to five minutes. The next step is to pour the liquid to be used (previously warmed) on to the diaphragm, in quantity sufficient to cover it. For twenty or thirty minutes, artificial respiration is maintained at regular intervals. The diaphragm may then be prepared as before for microscopical examination.

The Cellular Elements of the Centrum Tendineum in their Relation to the Lymphatic System.—The pleural surface of the *centrum tendineum* of a rabbit, guinea-pig, or any other small mammalian animal, is exposed as above described, and carefully, but slightly, brushed with a camel-hair pencil moistened with serum. Silver solution is then poured over it, and, after a few minutes, water. Thereupon bits are cut out for microscopical examination, which must be carefully separated from the parts in contact with their abdominal surfaces. These must then be mounted in glycerin, with the pleural side upwards. Immediately under the endothelium of the surface there exist large, flat cells, which are more or less branched. In the neighbourhood of the large vessels which pass through the *centrum tendineum* these are so close together that they are marked off from each other by mere lines of interstitial substance, and appear as if they formed a second layer of flat endothelial elements subjacent to the one brushed off. Under these cells branched cavities are seen to exist, hollowed out in a yellow or yellowish-brown ground-substance. When the examination is made with sufficient care, it is found, first, that each of these cavities contains a nucleated mass of protoplasm, which completely occupies it; and, secondly, that both the cavities and their contents are in continuity with each other, so as to form a network. This network of cavities was first described by Recklinghausen, under the name of *Saftcanälchen.* We propose to call it *lymphatic canaliculi,* and the more or less branched cells contained in them, lymphatic cells.

If these are examined in an island of tissue surrounded by lymphatic capillaries, it is seen that there are places in which the cells are closer together and less branched than in others, and that in such spots they are often arranged in linear series, or in small groups, each cell being marked off from its neighbours by interstitial lines, so that they resemble an endothelium. This is particularly the case in the immediate neighbourhood of the lymphatic capillaries; and here it can often be made out that cells contained in canaliculi are in contact with the elements of the lymphatic endothelium. The canalicular cells are also in communication with the flat cells which form the layer immediately covered by the serous endothelium.

The anatomical relation of the canaliculi and lymph cells on the abdominal side are the same as in the pleura. To demonstrate them, the abdominal surface of the *centrum tendineum* is prepared in the same way as last described.—In the neighbourhood of the large vessels, the existence of a similar layer of flat elements lying underneath the serous endothelium, resembling in character those already described in the pleura, can be demonstrated. The canaliculi and lymphatic cells of the *propria* have also the same relation to each other and to the superficial network of lymphatic capillaries as in the pleura. Finally, it is to be mentioned that in the tissue which occupies the fascicular channels which contain no lymphatics, the same character can be observed. Here the continuity of the lymphatic cells with the similar cells of the tendon-tissue, which, as we have seen, are oblong branched placoids, and apply themselves to the surfaces of the primitive bundles of fibrils, can be demonstrated.

Pseudo-Stomata.—In examining carefully the pleural surface of a diaphragm which has been treated with nitrate of silver, without pencilling, it is seen that there are here and there spaces between the endothelial elements, which are occupied by bodies of a more or less branched contour. These are usually darker in colour than the endothelium of the surface, and often exhibit distinct nuclei. If the preparation has been partly pencilled, it is often possible to observe, at the junction of the pencilled and unpencilled part, that the bodies in question are of the same kind with the flat branched cells which are covered by the endothelium; and it can be also made out that, even where they are covered, the cells of this layer send up projections between the endothelial cells which reach the surface. These projecting cells may be called *pseudo-stomata*. The intimate relation which exists between the sub-endothelial cells and those of the propria, and between these last and those which line the lymphatic vessels, has been already referred to. That these cells are also concerned in absorption is indicated by the fact that, in chronic inflammation, and other conditions in which absorption from the serous surface is more than usually active, these pseudo-stomata and the canaliculi are the seat of germination—and that if colouring matter in a state of fine division has been previously introduced into the peritonæum, they are found to contain it.

Lymphatic System of the Omentum and Mesentery.—To demonstrate the lymphatic structures of the omentum, the peritonæal cavity is opened in a rabbit just killed. The large and small intestines having been pushed aside to the right, the omentum, which usually lies on the anterior surface of the stomach, is carefully brushed *in situ*, from below upwards with a camel-hair pencil, moistened with peritonæal liquid. Half per cent. solution of silver is then allowed to drop over the surface from a capillary pipette, until the membrane is distinctly turbid. It must then be gently streamed with water, and removed along with the stomach, and placed in water exposed to the light. Portions of the membrane are then cut out, and covered in glycerin with the pencilled surface upwards. In preparations so obtained, an abundant network of lymphatics presents itself. In addition to this, there are certain parts of the surface in which the lymphatic cells are crowded together in patches. It is seen, in parts where the endothelium has not been completely removed, that the cells which lie immediately underneath it project so as to form pseudo-stomata, and in other respects stand in the same relation to it and to those which line the lymphatic capillaries, as on the surface of the diaphragm. It is also seen that in some of the patches there are lymph sinuses which communicate with the surface by true stomata. As was indicated in Chapter II., the endothelium which covers these lymphatic patches, particularly that which surrounds stomata and pseudo-stomata, differs from that of the general surface, by the smaller size, polyhedral form, and granular appearance of the elements. The same tissue also presents itself in the form of tracts alongside of the larger vessels.

In the dog, guinea-pig, and cat, these tracts are particularly well developed along the large vessels which are to be found in the trabeculæ of the membrane. Connected with these, there are nodular structures which project more or less from the surface. Both the tracts and the nodules consist of aggregations of lymphatic cells close togther, richly supplied with blood-vessels, and covered with an endothelium which has the same characters as that which covers the patches. In the neighbourhood of the tracts, lymphatic vessels are usually to be seen on one or both sides, which often communicate by cross branches, and stand in the same relation to the lymphatic elements as in the patches.—The canaliculi of the tracts and nodules sometimes contain what appear to be young cells, which, from what has been observed in pathological conditions of the structure, must be regarded even in the normal state as to a great extent offsprings of the endothelial elements.

Mesentery.—In the mesentery treated in the same way as that above described, in addition to the lymphatic vessels which proceed from the intestine, numerous lymphatics with dilatations (sinuses) can be demonstrated. The canaliculi with which they are surrounded and in communication, pervades the ground-substance of the mesentery in every direction. In the cat and rabbit, the endothelium of the mesentery exhibits pseudo-stomata of the same character as in the omentum. In that of the toad and common frog, the trabeculæ, in which the large vessels run, split into a kind of meshwork of smaller processes, the spaces of which are occupied by large lymphatic sinuses, in which a beautiful endothelium with sinuous outlines can be demonstrated by the silver method.

Injection of Lymphatic Glands and of Mucous Membranes.—For the injection of the lymphatics of the lymphatic glands, and of the mucous membranes, the "*Einstich Methode*" (method of puncture) of Ludwig is the best. The liquids used are either half per cent. silver solution or Prussian blue. The animal to be employed must be perfectly fresh. A very fine glass cannula is used, which is connected either with a syringe or with the apparatus described in the last chapter. The method consists in penetrating any tissue in which there are numerous lymphatics (*e.g.*, the submucosa of the mucous membranes, the cortical substance of a gland, or the loose tissue beneath the costal pleura), with a needle to a sufficient distance. The needle having been withdrawn, the cannula previously filled with the solution to be injected is introduced into its track, and connected with the apparatus or syringe as the case may be. The cannula having been seized with ordinary dissecting forceps, the liquid is injected. If it is seen that the coloured liquid is not contained in vessels, but merely occupies a bulging cavity in the tissue, the injection must be discontinued as unsuccessful. In the case of the mesenteric or inguinal glands of small animals, I succeeded in obtaining good results with a tube drawn out at one end to a very fine point, and bent near the point at right angles. This tube having been filled with the liquid, was injected by the mouth. In the rabbit, nothing can be easier than to insert such a tube into one of the lymphatics of the mesentery, and in this way to inject the gland to which it leads.—Lymphatic glands, after injection, must be placed in alcohol and used for the preparation of sections.

Structure of the Lymphatic Vessels.—The structure of lymphatic vessels may be further advantageously studied in those of the mesentery. In small cats or rabbits, it is easy to prepare the parts of the mesentery in which there are abundant lymphatic vessels leading to the mesenteric glands, by stretching them on cork and treating them with silver, after pencilling away the endothelium of the peritonæum with a camel-hair pencil, moistened with serum. In these vessels, it is possible to demonstrate the existence of transverse muscular fibres.

Structure of the Lymphatic Glands.—*Mesenteric Glands.*—The best glands for the purpose of study are those of the calf, ox, or cat. Small portions can be placed in Müller's liquid, or in solution of bichromate of potash. After a few days, it is possible to make thin sections of small extent. It is easier, however, to make sections of glands which have been steeped two or three days in common alcohol. The sections, stained or unstained, are placed two or three together in a test tube, half full of water, which must then be shaken regularly but briskly until the sections acquire the characters of reticulated membranes. The contents of the test tube are poured into a shallow capsule, and prepared in the usual manner, either for mounting in Dammar varnish or glycerin. It is well not to continue the agitation longer than is necessary to get rid of the medullary substance of the gland. The globular or ovoid follicles which constitute the mass of the cortical substance, and are in continuity with the lymphatic cylinders, are seen in such preparations to have the following structure:—Each consists of a close network of fibres, the meshes of which are of nearly equal size. It is further seen that the fibres are thickened at the nodes, and that each thickening contains a nucleus. The younger the animal, the more obvious it is that the network consists of branched cells. The follicle also contains numerous capillaries. From the network of branched cells which forms the adventitia of each capillary, spring filaments which either stretch to neighbouring capillaries, or form a part of the general adenoid network of the follicles. These filaments are always broader at their bases than elsewhere, and have thickenings which contain nuclei. In sections which have not been agitated, the whole .network is filled with small roundish bodies (so-called lymph corpuscles). It can be readily shown in glands which are injected from the lymphatics, that each follicle is surrounded by sinuses, which are mere dilatations of the different lymphatic vessels of the cortex, and are in like manner lined with endothelium, as is seen in glands injected with nitrate of silver. Outside of the follicles is a layer of connective tissue which contains numerous blood-vessels, and is continuous towards the hilus, with the trabeculæ, which form the framework of the organ. Outwards it is intimately united with the capsule. In glands of which the blood-vessels are injected, capillary loops can be seen to penetrate into the follicles from the rich network of blood-vessels with which each is invested. The part of the gland between the cortical substance and the hilus consists of lymphatic cylinders, and intervening trabeculæ. The former are united with each other so as to form a network, and have the same intimate structure as the follicles, as regards the adenoid network, the cells it contains, and the capillaries. In general they possess only capillaries; occasionally, however, larger vessels enter them. The trabeculæ consist of fine fibres which run mostly parallel to each other; they are connected into a meshwork, the intervals of which are occupied by the network of cylinders. There are, however, between the outer surfaces of the cylinders and the trabeculæ, spaces to which we shall revert immediately. In sections which have been only slightly shaken, it is possible to observe that fibres stretch at more or less regular intervals from the external surface of the cylinders to the trabeculæ, by which the intervening space is divided into sections. These fibres appear to be offsets from the trabeculæ, and exhibit either swellings containing nuclei, or distinct nucleated stellate cells. In sections which have not been shaken, the whole medullary substance appears to be uniformly full of lymph corpuscles. In the spaces, as we have seen, they can be shaken out so readily, that it is evident they lie quite loosely; whereas, in the cylinders themselves, they are intimately united to the network. The significance of this structural difference can be demonstrated in glands in which the different lymphatics have been injected, or, still better, in glands which have been injected by the puncture method with nitrate of silver. In the former case, it is possible to trace the injection from the different vessels of the cortex, through the lymph sinuses which surround the follicles to the spaces which separate the cylinders from the trabeculæ, and thence to the different vessels at the hilus. In the latter case, lymphatic sinuses are met with in the medullary substance near the hilus, lined with endothelium, which are continuous with the spaces surrounding the cylinders in such a way that their endothelium can be distinctly traced on to the surface of the trabeculæ.

Solitary and Agminated Follicles of the Intestine.—Follicles such as we have just described in the cortical substance of the lymphatic glands occur in the large intestine as solitary follicular bodies, or in the small intestine, in groups (the so-called Peyer's patches. *See* p. 125).

Thymus Gland.—This is to be regarded merely as an aggregation of follicles of the same kind. Neither in their structural elements, nor in the relation of these to the vessels or lymphatics, can any difference be made out. In man, as well as in the dog, the external surface of the capsules is covered with an endothelium identical with that of the pleura. The tonsils and follicular glands at the base of the tongue are also made up of aggregations of lymphatic follicles.

CHAPTER IX.

ORGANS OF RESPIRATION.

The structure of the larynx, trachea, and bronchi can be completely studied in sections of organs hardened in chromic acid. The epithelium has been already fully described elsewhere. An animal having just been killed, the tubes are opened, washed with very dilute solution of bichromate of potash, and placed in the hardening liquid. In thin sections, the relations of the mucosa, submucosa with its glands, cartilages, perichondrium, muscular fibres, and ganglia, may be completely made out. The blood-vessels may be injected in the ordinary way, and the lymphatics by puncture of the submucosa. The network of elastic fibres which surround the alveoli are most readily studied in thin sections of fresh-frozen lungs of small mammalia. The sections are steeped in acidulated water till the air-bubbles have escaped, and then spread out on an object-glass and covered in glycerin. The structure of the fine bronchi may be well studied as regards its epithelium, minute glands, muscular coat, and innumerable large ganglionic masses, in sections of lungs of human fœtuses of the last months of pregnancy, which have been hardened in one-tenth or one-eighth per cent. solution of chromic acid. The flat epithelial elements of the alveoli, as well as those which line the finest bronchial tubes, can be best examined in lungs of small mammalia, prepared by placing a cannula in the trachea, removing the sternum, and then injecting the bronchi with one-tenth to one-eighth per cent. solution of chromic acid, until the organ is moderately distended. The trachea is then tied, and the lungs are carefully removed from the thorax along with the heart, after separating them first from the spinal column, and then from the diaphragm ; the whole is then placed in liquid of the same strength. Another method, which, however, does not answer so well, is that of injecting half per cent. solution of silver into the pulmonary artery. Good injected preparations of lungs can be obtained by filling the air-passages with cacao butter, and the blood-vessels with gelatin-mass, simultaneously. A rabbit is killed by opening the crural artery. The trachea having been prepared, a cannula fitted to a nozzle is fixed in it. The sternum is then removed, and a second cannula inserted in the pulmonary artery close to its origin. As much air as possible is now pumped with a syringe out of the trachea, and the stop-cock closed. The apparatus for injection having been previously put in readiness, all must be connected, and the pressure raised in the tube to the required point, *i.e.*, sixty to eighty millimeters, so that at any moment the stop-cock of the nozzle may be opened and the injection begun. A sufficient quantity of cacao butter having been fused in a capsule, a middle-sized syringe is filled with the hot liquid, and fitted into the nozzle, which is inserted into the trachea, and the injection begun. The moment that the cacao butter has begun to enter, an assistant opens the stop-cock* of the cannula in the pulmonary artery. As soon as the lung appears to be distended with the butter, the stop-cock of the trachea is closed, but the injection of the blood-vessels is continued. As soon as this appears to be complete, the left auricle of the heart is comprised in a ligature, by tightening which the pulmonary veins are completely closed. A few moments later, the stop-cock in the artery is also closed, and the animal placed in a basin so that the thoracic organs are immersed in cold water. As soon as the lungs are seen to be firm, they are taken out with the trachea and placed in common alcohol. In two or three days, small portions may be cut out and placed for a short time in absolute alcohol, and then embedded for the preparation of sections. The sections must be steeped in oil of turpentine or cloves, till the cacao butter is dissolved out; this may be ascertained by placing the watch-glass containing them under a low power. Turpentine accomplishes this more quickly than oil of cloves. The sections must be mounted in Dammar varnish. The relation between the different blood-vessels and the capillary network of the walls of the alveoli, are admirably seen in such preparations. If it is intended to preserve the structure of the pulmonary tissue unimpaired and at the same time to inject the blood-vessels, half per cent. solution of salt or of bichromate of potash may be substituted for the cacao butter, and two per cent. solution of Prussian blue for the gelatin mass. The organ must be placed in alcohol as before.

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* Great care must be taken to keep the tube leading from the bottle containing the mass, as well as the nozzle, warm with hot sponges, otherwise there will be great danger of the solidification of the gelatin in those parts, during the time which intervenes between the preparation of the apparatus and the commencement of the injection.

CHAPTER X.

ORGANS OF DIGESTION.

Teeth.—Polished sections of teeth are prepared in the same way as those of bone. They must be made in various directions. For the study of the development of the teeth, maxillary bones of human fœtuses, softened in chromic acid in the way previously directed, must be used. The reader is referred for the description of the structure to the ordinary handbooks of general anatomy.

Salivary Glands and Pancreas.—These organs must be steeped several days in half per cent. solution of bichromate of potash and prepared by teasing. Small bits of the fresh glands may be steeped for forty-eight hours in the dark, in one-tenth to one-half per cent. solution of osmic acid, and then either placed in water for a day or two, with a view to preparation by teasing, or hardened in alcohol for the preparation of sections. In either case the preparations, if kept, must be placed in concentrated solution of acetate of potash. The arrangement of the alveoli and their ducts, and the characters of the epithelium of each, can be best seen in sections of glands hardened in alcohol, and stained with dilute carmine. In such sections, the beautiful mosaic of the polyhedral epithelial cells of the alveoli, each consisting of granular protoplasm, forms a striking contrast to the cylindrical epithelial lining of the ducts; the latter consisting of pale slender cells, each of which appears streaked in the direction of its length, and contains an oblong nucleus in its outer third. The alveoli are united into groups (lobules) by delicate bundles of connective tissue. In teased preparations, the cellular and fibrous elements of the connective tissue, and the ganglion cells which are met with here and there, can be studied. In injected glands, each alveolus is seen to be invested by a delicate and very abundant network of capillaries.

Mucous Membrane of Mouth, Tongue, Pharynx, and Œsophagus.—The structure of these mucous membranes can be well seen in sections of organs hardened in chromic acid.* For studying the epithelium, the papilla, the glands, and muscles, this mode of preparation is sufficient. The lymphatic vessels, *e.g.*, in the pharynx and at the root of the tongue, can be filled by the puncture method, after which the injected parts must be hardened in alcohol, and used for the preparation of sections. To see the loops of fine capillaries in the papilla of the mouth, tongue, and pharynx, these parts must be injected. As regards the distribution of the fine nerves, *see* Chapter VIII.

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* As regards the tongue, *see* also the chapter on Organs of Special Sense.

Stomach.—The relation of the *muscularis mucosæ*, the submucous tissue, the musculosa and the ganglia to each other, can be well shown in sections of organs hardened in chromic acid. For the study of the glands, the best method is to open the stomach of the cat or dog immediately after death, carefully inverting it so as to empty it of its contents, and then to stream it gently with water. Thin folds of the membrane must be snipped off with sharp curved scissors and placed in common alcohol. After three or five days the objects are ready for the preparation of sections, the direction of which must be parallel or vertical. The parallel sections must be made at various depths. For the colouring of these sections a staining liquid prepared after Beale's formula (omitting the alcohol) answers well; but it is necessary to free it from excess of ammonia, either by careful neutralization with acetic acid or by warming it in the water-bath. The sections having been placed in this liquid in a watch-glass, it is put in a closed vessel along with a second watch-glass containing water with a trace of ammonia. After twenty-four hours the sections are removed, washed in dilute glycerin, and transferred to concentrated glycerin in another watch-glass, which is then placed in the closed vessel along with a glass containing common acetic acid. After twenty-four to twenty eight hours the sections may be finally covered in glycerin. In such preparations the gland tubes of the fundus (the so-called peptic glands), with their two kinds of epithelium, are well seen. Next the cavity of the gland it consists of cylindrical cells (the *Hauptzellen* of Heidenhain), which are scarcely coloured by the carmine, and are very finely granular. The nuclei of these cells are occasionally coloured, but usually not so. Underneath them, *i.e.*, next the *membrana propria*, both in vertical and parallel sections, ovoid granular cells are seen which are strongly stained. These last (the *Belegzellen* of Heidenhain) do not form a continuous layer in either direction: they occur in small numbers in the half or two-thirds of the gland next the *muscularis mucosæ*, *i.e.*, in the body of the gland—more abundantly in the adjoining part, which is usually called the neck, where they more or less conceal the cylindrical layer. The short duct, in which usually two tubes open, possesses an epithelium of the same kind as that which covers the surface, consisting of slender cylindrical elements.

When these structures are compared as seen in fed animals and in animals in inanition, it is found that in the former the staining extends both to the ovoid cells and to the columnar cells. A difference of the same kind may be shown in similar sections stained with anilin. An extremely dilute aqueous solution is used. The sections must be placed in a watch-glass containing the liquid, which is allowed to stand twenty-four hours in a closed vessel, the air of which is kept saturated with moisture. The preparations can then be at once enclosed in glycerin. The only difference between the results of the two methods is, that the cylindrical cells are here slightly tinged even in inanition. The convoluted and much-branched tubes which occur in the region of the pylorus contain only cylindrical cells, which correspond to those of the same form in the proper peptic glands. Between these last and the branched glands, intermediate forms are met with, which differ from each other in the number of ovoid cells (*Belegzellen*) which they contain, the number diminishing according to the distance from the pylorus.

The processes of the *muscularis mucosæ,* which stretch towards the surface through the *mucosa* between the glands, can be better seen in chromic acid preparations.

Small Intestine.—The characters of the epithelium of the small intestine in the fresh state have been already described. They may be further advantageously studied in sections of hardened organs, which will also serve for the demonstration of the following structures—the dense reticulum of the substance of the villi, with the round cells in its interspaces; the anatomical relations of the single or double central lymphatic vessel which each villus contains; the slender bundles of longitudinal unstriped muscular fibres which run out around the lymphatics towards the apex of each villus; the reticular tissue of the mucosa, identical in its characters with that of the villi, in which the tubes of Lieberkuhn are sunk; the *muscularis mucosæ,* with the distinct layers of which in many parts it is seen to consist, and the bundles of fibres which extend from it, either towards the villi or between the glands; and, lastly, the *submucosa* and *muscularis externa.* The intestine should be treated as follows:—The intestine of a cat, dog, rabbit, rat, or hedgehog just killed is opened, small portions are at once placed in water coloured with bichromate of potash, and washed. They are then transferred to a one-tenth or one-eighth per cent. solution of chromic acid, and five or six days later to dilute alcohol, in which they are steeped for some days. Thereupon small portions are embedded in gum, and coloured and mounted as directed in Chapter VI.

The Glands of Brunner.—These glands may be studied in thin vertical sections of the duodenum of the cat or dog. They lie in the submucosa, and consist of branched tubes, which are much convoluted and are lined throughout with cylindrical epithelium. Towards the *muscularis externa* they are invested by a special layer of unstriped muscular fibres, originating from the *muscularis mucosæ*. The ducts of these glands, after penetrating the *muscularis mucosæ*, diminish in calibre as they pass outwards towards the surface between the Lieberkuhn's tubes. The epithelium with which they are lined exhibits a striking contrast to that of the tubes, the elements being slenderer and much more readily stained with carmine.

Peyer's Follicles.—The best preparations are to be obtained from the lower end of the ileum of the dog or cat. The intestine of the rabbit may also be used. Thin sections of these parts may be prepared as above directed, with the exception that the time occupied in each stage of the process of hardening may be shortened. The hardened portions must, moreover, be embedded in wax-mass rather than in gum. The sections, whether stained or not, should be steeped for twenty-four hours in water, and then shaken in the manner recommended for the preparation of sections of the lymphatic glands. They are finally mounted in glycerin. In this way the reticular tissue both of the mucosa and of the follicle is well shown. From sections of Peyer's patches, prepared in the manner previously described, we learn that each follicle is surrounded by a large lymphatic sinus—that each is deeply embedded in the submucosa, sometimes approaching the *muscularis externa*—that a small part of each penetrates the *muscularis mucosæ* and projects into the *mucosa*, some of the summits losing themselves in its tissue without any defined limit, others reaching up to the epithelium. When this is the case, the epithelial elements are smaller, and consist of several layers of polyhedral cells. Both in situations where there are distinct patches, and in those regions in which (as occurs in the ileum of the cat and dog) the whole of the *submucosa* is occupied with follicles, the individual follicles are in continuity at their widest part. The network of lymphatic vessels of the submucosa, with which the sinuses of the follicles, as well as the lymphatics of the villi, are in immediate communication, can be readily filled with soluble Prussian blue, by the method of puncture. It is most easily accomplished in large rabbits. Half per cent. silver solution may be also used for the demonstration of the endothelial lining which all these vessels possess.

To prove that in the absorption of fat the network of the stroma of the villi is concerned, a rat, hedgehog, or kitten is allowed to remain without food for a day or two, and then fed with milk (rat, kitten) or fat meat (hedgehog), and killed a few hours afterwards by strangulation. The belly having been opened, those parts which to the naked eye appear best filled are ligatured without delay, and placed at once (without opening them) into Müller's liquid, previously slightly warmed. After a few days, small portions are cut out and immersed in half per cent. solution of osmic acid, and then, twenty-four hours later, replaced in Müller's liquid, or in ten per cent. chromic acid solution. Bits of the intestine so prepared must finally be embedded in gum-mass for the preparation of sections, which must be mounted in acetate of potash. In sections which comprise villi, the epithelium, and the reticulum and central lymphatic vessel of each villus are observed to be filled with fat drops stained brown or black by the reagent. When a villus is cut transversely, it is seen that trabeculæ beset with blackish or dark brown fat drops, arranged in a reticulate manner, radiate from the central lymphatic outwards to the epithelium.

Blood-Vessels.—The arrangement of the capillary networks which surround the glands, and those of the villi, must be studied in injected preparations.

Nerves.—Meissner's and Auerbach's ganglia have been already referred to sufficiently in Chapter V.

Large Intestine.—The methods for studying the epithelium, the Lieberkuhnian tubes, and the solitary follicles of the submucosa, the mucosa and muscular structures, are the same as those used for the small intestine. The agminate follicles, with their lymphatic sinuses, may be particularly well seen in the vermiform appendix of the rabbit. The muscularis and glands of the mucosa are best seen in the wart-like prominences of the colon. Good examples of the Lieberkuhnian tubes, the *muscularis mucosæ,* and the solitary follicles, are to be obtained from the dog. The ganglia of Meissner are well seen in the dog and cat, and in the human fœtus.

Liver.—For the study of the liver, fine sections of the fresh organ may be employed. By teasing these out with needles, the characters of the elements of the connective tissue, and the form of the liver-cells and their nuclei, can be satisfactorily made out. The arrangement of the cells in the acini can be demonstrated in sections of liver of human fœtus, or of the smaller domestic animals, hardened in solution of bichromate of potash or very dilute solution of chromic acid. The best plan is to steep very small portions of liver for four or five days in a large quantity of one to two per cent. solution of bichromate of potash, and then for twenty-four to forty-eight hours in common alcohol. The sections so obtained are stained in the usual way in carmine.—In such preparations the beautiful regular groups or oblong tracts of liver-cells, with the capillaries which separate them from each other, are well seen. Here and there it is observed that an interstitial hole or orifice appears to be formed by the apposition of two semicircular notches in the border of contiguous cells, or, in other cases, by three cells similarly notched. By comparing these appearances with sections of organs in which the ultimate bile ducts are injected, it is seen that the orifices correspond to sections of these channels. They possess no special wall, being apparently bounded immediately by the cell-substance. In such preparations the cylindrical epithelium of the interlobular ducts can also be well seen. The blood-vessels should be studied in organs in which the *vena portæ* has been previously injected with gelatin mass; for which purpose the liver of a rabbit, guinea-pig, or small dog, answers best.—The animal having been killed by bleeding, a cannula is inserted in the vein, and a ligature placed round the *vena cava* in the thorax. Before injecting the mass, it is best to send warm half per cent. solution of salt through the organ, till it becomes colourless. Carmine-gelatin or Prussian-blue-gelatin mass must then be injected in the manner directed in Chapter VI. Before leaving off, the ligature on the cava is tightened, after which a somewhat stronger impulse is given, so as to keep the vessels distended. The *vena portæ* having been ligatured, the organ is treated as before directed. In such preparations the whole course of the vessels from the interlobular veins, through the capillary system of each acinus to the intralobular vein, may be studied. If it is desired to inject the hepatic artery and the portal system with different colours, this may be accomplished by securing a cannula at the same time in both vessels; the nozzle of the one cannula being connected with a Woolff's bottle containing carmine mass, that of the other with a similar bottle containing Prussian blue. The connecting tubes leading to the two bottles are adapted, one to each arm of a T tube, the stem of which is in communication with the pressure apparatus, so that the same pressure is exerted at the same time in both bottles. The bile ducts can be injected naturally by the same method which is used for injecting the urinary tubes, or in the ordinary way by the hepatic duct. After ligaturing the cystic duct, two per cent. solution of Prussian blue can be injected with such success that in parts the capillary bile ducts are filled. The livers that answer best for the purpose are those of mature fœtuses, puppies and rabbits. As soon as a successful injection has been obtained (as may be judged of by inspection), it is desirable to inject the portal system with a different colour.

The Spleen.—For the study of the elements of the pulp of the spleen it is absolutely necessary to use the organs of animals just killed. Preparations may be made either by scraping the sectional surface, or by teasing. The tissue of the trabeculæ, the special sheaths of the arteries, the stroma of the pulp and that of the Malpighian corpuscles are best studied as follows:—Small bits of fresh spleen are steeped in one or two per cent. solution of bichromate of potash till they are fit for making sections. The thin sections are then washed in water (after colouring if desired), and carefully shaken in a test tube. They are then covered in glycerin. In organs successfully injected and prepared in the usual way, it can be made out that the vascular system is not definitely limited as in other tissues. The circulating blood, before reaching the veins of the pulp, passes through a system of channels without definite walls, the so-called *vasa serosa.*

CHAPTER XI.

SKIN, CUTANEOUS GLANDS, AND GENITO-URINARY APPARATUS.

Section I.—Skin.

Methods of Study.—For the study of the structure of the skin in general, the human integument is preferable to that of the lower animals. Portions of skin with subcutaneous cellular tissue, obtained in as fresh a state as possible, are placed in sherry-yellow solution of chromic acid, containing from one-tenth to one-fourth per cent. After a week, or even sooner, they should be transferred to common alcohol and used for the preparation of sections. As regards examination of the epidermis, it is only necessary to add to the directions given in Chapter II., that the best parts of the skin for the preparation of sections are the volar side of the fingers, the lips, the alæ of the nose, and the eyelids. Any part will answer equally well for the investigation of the structure of the corium. If it is desired to demonstrate the sweat glands, the palm of the hand, the axilla, and after these the forehead, answer best. Hairs can be examined in the skin of the scalp, the upper lip and eyelids. The sebaceous glands, whether those which open into hair follicles, or those of which the orifices are free, can be best prepared in the *labia majora*, prepuce, scrotum, or internal lining of the orifice of the nose or eyelid of new-born children, and in the scalp of adults. The unstriped muscular fibres of the skin, particularly the hairs, can be studied in the scalp and scrotum, or in the skin which covers the anterior and external aspect of the thigh. The blood-vessels can be best studied in injected preparations, for which purpose the best way is to inject one of the upper extremities of a new-born fœtus.

The lymph vessels can be made out most easily in œdematous skin. The integument must be removed with the whole of the subcutaneous tissue, and then scarified at one or two points, and left twenty-four hours suspended, until much of the liquid has drained away. The vessels can then be injected by the puncture-method.

The preparation of the nerves and cellular elements of the corium and papillæ by the gold method has been already described. The Pacinian corpuscles and tactile corpuscles of Meissner can be advantageously seen in thin sections of the volar side of the finger or palm, after hardening in chromic acid.

Sweat Glands.—The sweat glands are of two forms. Those of the first form are long and slender tubes closed at one end. The secreting part, or body of the gland, is convoluted, and is embedded in the subcutaneous tissue at a variable depth; the duct which passes through the corium to the surface, follows a slightly winding course. The gland, whether seen in transverse or longitudinal sections, is found to be limited by a fine membrane (*membrana propria*) lined by a single layer of cylindrical epithelium, the free surface of which forms the internal surface of the gland. In very thin sections, in which it is possible to compare the epithelium of the ducts with that of the body of the gland, it is seen that the elements of the former are more slender. In the duct it is further noteworthy that the nucleus of each element is in its outer third, and that the nuclei are regularly arranged. In the elements of the body of the gland they lie in the middle of each cell. In the epidermis, the duct is continued towards the surface as a canal, which winds spirally, like a corkscrew. This is particularly the case when the epidermis is of some thickness.

In a section which shows the whole course of the canal, it is seen that the *membrana propria* becomes continuous with the most superficial layer of the corium, while the epithelium of the duct becomes identified with the elements of the *rete Malpighii*. This first form of sweat glands is met with over the whole integument. The glands of the second form occur along with the others in grown persons only, and are subject to great differences as regard their distribution. They are always to be found in the skin of the palm of the hand, of the axilla, and of the scalp. They are met with in some persons in other parts of the body. They are distinguished from the common form by the facts that they are three or four times as large, that the tube is as much wider, and that the epithelium consists of larger elements, which are coarsely granular, and of polyhedral form, and occasionally contain yellowish-brown pigment.

As the epithelium elements are often found separated from the *membrana propria*, it may be inferred that they are much more loosely attached to it than in the other form. Further, it is to be noticed, that the *membrana propria* contains a continuous longitudinal layer of unstriped muscular fibres, which seem to lie towards its inner surface. Wherever glands of this form occur, they appear to be quite distinct from the others, for no intermediate or transition forms present themselves. It is possible that these glands have a casual relation to the offensive odour of perspiration in certain persons.

The orifices of the ducts of both kinds of sweat glands are lined with laminated epithelium, which is in direct continuity with the *rete Malpighii*. The cells of the layer which lies on the propria are of a polyhedral or rather pallisade form. Those which lie next them are somewhat flattened, forming layers which are more and more scanty the further they are from the orifice; they entirely cease where the duct joins the gland. The *membrana propria* of the gland itself is lined by a layer of polyhedral cells, which are of uniform size and appearance, and consist of protoplasm. These are readily stained by carmine, and are continuous with the deepest layer of the epithelium (the pallisade cells).

Sebaceous Glands.—The sebaceous glands consist of closed tubes, which are usually branched, and receive a variable number of tributary sacculi. They either open at the surface, or into hair follicles. In every sebaceous gland the secreting part may be distinguished from the duct. The duct is lined with pavement epithelium, which, when the orifice is at the surface, can be seen to be continuous with the *rete Malpighii*. In glands which open into hair follicles, it is continuous with the external sheath of the bulb. In passing from the duct to the secreting part, the epithelium changes its character, being represented by a layer of granular, cubical, or polyhedral elements, which lines the propria. Besides these cells, the sacculi contain larger elements, which are so closely packed together as to be flattened against each other. In fresh preparations these appear to be loaded with fat, but in preparations treated with absolute alcohol and oil of cloves they exhibit a distinct nucleus and investing membrane. The sebaceous glands can be best studied in the skin of mature fœtuses, *e.g.*, in that of the lips and nasal orifice, *labia majora*, prepuce, and scalp. The acinous form is exemplified in the Meibomian follicles of the eyelids. Sections of these parts hardened in chromic acid must be made, which can be stained and mounted in Dammar varnish.

Hair.—With reference to the structure of hair, it is of importance to notice that each follicle consists of a connective tissue layer, and of a layer of muscular fibres. The former, which is richly supplied with capillaries, is formed of fibres which run mostly longitudinally, and seem to be merely a condensation of the surrounding tissue. In certain parts, this layer is in immediate contact with the external hyaloid membrane of the hair; in others, there exists between them a circular layer of plain muscular fibres, which varies in distinctness in different varieties of hairs, but is always most strongly developed in the neighbourhood of the bulb. In the eyelash of the mature fœtus, the muscular layer is much stronger than the connective tissue layer, and can be traced over the whole of the bulb. As regards the structure of the hair itself, all that is required will be readily understood from the description given in the ordinary text-books.

The structural facts relating to the root of the hair can be easily made out in chromic acid preparations. The structure of the shaft can be best seen by preparing fresh hair (of the scalp) in concentrated acetic acid, by which means the cuticle and the elements of the medulla are brought into view. For the isolation of the plates of the cuticle, and of the fibre-cells of the substance of the hair, concentrated sulphuric acid is used, at a temperature of 40° to 50° C., in which the hair must be heated for about an hour. After steeping for several days in two per cent. solution of caustic potash, the elements of the medulla become very distinct. The development of the hair, and of the sweat glands and sebaceous glands, may be studied in embryos at various periods, in preparations hardened with chromic acid. The most important point to notice is, that in mature embryos, or even in the eyelashes of children, if the section coincides precisely with the axis of the hair and involves the papilla, it is seen that that part of the external hyaline membrane which extends over the papilla is uninterruptedly covered with the regularly arranged cells of the external sheath, and that these cells occupy the whole bulb to about half-way up the root. It is common to find several stages of development in a single preparation, from which it can be learnt that the new hair takes its origin from the axial cells of the sheath of the root, being formed by the lengthening of these elements.

Section II.—Urinary Apparatus.

Epithelium of the Kidneys.—For the study of the epithelium of the kidneys, the pig, dog, or mature fœtus may be used. The fresh kidneys having been divided into two halves, in the direction of the length of the organ, juice from the cut surface may be employed for the study of the epithelium of different parts. It is, however, better to cut one of the halves transversely into a number of parts, which may be placed in a large bottle filled with half or one per cent. solution of bichromate of potash. After from eight to ten days, sections of the cortex are prepared, as thin as possible. Some of these must be made in the direction of the pyramidal processes (which are readily seen by the naked eye), others at right angles to these processes, and parallel to the surface. Other sections comprising as much of the medullary substance as possible, must in like manner be made in both directions. The cross sections should be taken from various parts of the medullary substance, some comprising the papillæ, others the intermediate part. The sections having been washed in water for fifteen minutes or more, may be either mounted at once in glycerin, or after previous staining for twenty-four hours in diluted solution of carmine. Such preparations show the characters of the epithelium in the tubes throughout their whole extent, and in the loops of Henle. It may be further seen that in many of the convoluted tubes of the cortex, the uniformly granular substance can be distinguished into distinct polyhedral cells, each possessing a spheroidal nucleus. By teasing the sections obtained as above, it is possible to isolate straight tubes or loops, but this can be better accomplished by another method to be described further on.

Epithelium of the Malpighian Capsules.—For the demonstration of the epithelium which lines the internal surface of each Malpighian capsule, and the surface of the glomerulus, it is best to employ kidneys of mature or immature human fœtuses. With this view the organ (which must be as fresh as possible) must be divided into small portions, and first placed for three to six days in one per cent. solution of bichromate of potash, and then transferred for one or two days into one-fourth to one-eighth per cent. solution of chromic acid. The sections are prepared in the ordinary way after embedding. In such preparations it is seen that the capsule of the glomerulus, which is characterized by its oblong nuclei, extends continuously over it, and that it is lined with a continuous layer of elements which are mostly cubical, but sometimes columnar. The epithelium of the convoluted tubes consists, in the human fœtus, of spheroidal or cubical cells. If a very small strip of the fresh kidney of the frog is prepared in salt solution or serum, it is seen that the epithelium, as well of the capsule as of the commencement of the convoluted tube leading from it, is beset with cilia of extraordinary length.

Isolation of the Tubes.—Long slices of fresh kidney so cut as to include both cortical and medullary substance, and to extend from the surface to the papillæ, are placed in a flask containing a mixture of eight parts of common alcohol, and two parts of hydrochloric acid. The flask is fitted with a cork, through which a very long glass tube passes. It is kept boiling for some hours, after which the liquid is poured away, and replaced by distilled water. In this liquid (which should be changed once or twice) the portions of kidney are steeped several days. They are then agitated in a test tube, containing a little water, by which means the tubes readily separate from each other. They can now be prepared in the same liquid for microscopical examination, or allowed to subside, and then separated from the liquid and mounted in glycerin. Pure hydrochloric acid is also used for the same purpose. The slices of kidney, which must be taken from an animal killed the day before, are steeped in hydrochloric acid of 1·120 sp. g., for five to twenty hours. Thereupon the portions are carefully washed with distilled water. Of these methods, the former is easier. By either it can be shown that the capsule of the Malpighian body is first contracted, and then dilated so as to form the convoluted urinary tubes, which are filled with a substance, the division of which into cells is almost indistinguishable. These tubes are continued onwards, first as the narrower descending limb of the Henle's loop, and then as the somewhat wider ascending limb. The latter again dilates, so as to form the intercalated convoluted tube (*Schaltstück*) which ends in a straight collecting tube. These last form the pyramidal processes, and unite finally into single ducts, by repeated junctions with each other at very acute angles.

The whole system of ducts may often be injected from the ureter. Injections can, however, seldom be carried beyond the loops. The most suitable kidneys for the purpose are those of the pig, dog, or rabbit. The animal must, if possible, be killed by bleeding. A cannula having been secured in the ureter, close to the point at which it leaves the pelvis of the kidney, two per cent. solution of Prussian blue is injected, under a pressure of from 60 to 100 millimeters. The ureter having been ligatured, it is desirable to fill the artery with carmine gelatin. The urinary tubes can be also injected during life by what is called the natural method. A rabbit of moderate size is allowed to lose 10 c.c. of blood from the jugular vein, replacing it with a filtered solution of carmine, containing two drachms of carmine, and one drachm of liquor ammoniæ in an ounce of water. If a dog of moderate size is used, 25 c.c. are required. Immediately after the injection, the ureters are ligatured, and the animal is allowed to live for an hour, and then killed. The blood-vessels are then injected with solution of Prussian blue in gelatin, and the organ is placed in common alcohol containing a drop or two of glacial acetic acid. Before placing the kidneys in alcohol, they must be steeped for a short time in concentrated solution of chloride of potassium. Instead of the carmine, solution of sulpho-indigotate of soda, saturated in the cold, may be used in exactly the same manner. The blood-vessels must, however, be subsequently injected, not with Prussian blue, but with carmine gelatin.

Pelvis, Ureter and Bladder.—The laminated epithelium of these parts may be studied in bichromate of potash preparations.

For sections, the membrane must be hardened in chromic acid. The methods for the study of the epithelium, muscular tissue, nerves, and ganglia, &c., have been already fully described in Part I.

Section III.—Genital Organs.

Epithelium and Endothelium of Ovary.—It has been recently shown by Waldeyer, that the ovary is only partly covered with peritonæum. Where this is the case the surface is covered with endothelium. The remainder of the surface possesses a cylindrical epithelium, to which the term germinal epithelium is applied. This can be demonstrated in the ovaries of the sow, bitch, and cat, and in the human ovary. In the last it can be seen both in the mature fœtus and in the adult. In the fresh ovary the line of demarcation can be made out, even with the naked eye. By scraping the surface with a scalpel, shreds can be obtained which may be at once prepared in salt solution. In those taken from the peritonæal part, large endothelial plates can be shown, each containing an oblong nucleus. In those from the other part, cylindrical cells are seen, which consist of distinctly granular protoplasm, and contain an ovoid nucleus and nucleolus. These possess the character of epithelial elements. If an ovary is placed a few minutes in silver solution and then washed the usual way in water, and hardened in alcohol, sections parallel with the surface of both parts may be prepared. In such sections, if made close to the surface, and covered in glycerin, the contrast between the two forms of cellular investment can be completely demonstrated.

The anatomical relations of the germinal epithelium, and of the *tunica albuginea*, stroma, and Graafian vesicles must be studied in sections. For this purpose the fresh organ obtained from any of the above-mentioned animals must be steeped in one or two per cent. solution of bichromate of potash for periods varying from four days to a week; it must then be transferred for a day or two to one-eighth or one-tenth per cent. solution of chromic acid, and can afterwards be kept in common alcohol. Small ovaries, such as those of mature fœtuses, or of other young animals, can be embedded *in toto*. Larger organs must be divided.

Stroma.—The most important peculiarity to notice is the extraordinary frequency of bundles of spindle-shaped cells which run cross each other in various directions. Their claim to be regarded as muscular or connective tissue cells is still open to question. Both in the sow and bitch, there are bundles of unstriped muscular fibres, which, along with blood-vessels, run from the medullary part into regions in which large follicles are to be met with, and form an investment of the follicular wall. There are, however, many bundles in the cortical substance which present no such definite characters. But in the ovary of the guineapig, muscular bundles can be distinctly recognized even in the stroma of the cortex.

Graafian Follicles.—The structure of these follicles can be made out completely in the preparations above referred to. For the study of their development, human fœtal ovaries and those of the dog must be used. In the former, it is seen that from that part of the surface which is covered with germinal epithelium, blind tubes are sunk to various depths and in various directions. These tubes are lined with an epithelium, which is continuous with that of the surface, and identical with it. It can further be made out that certain individual elements of this epithelium have a special character, being more readily stained with carmine, and that they are larger than the others. Between these and ovules all transitions can be observed. By the segmentation of a single tube into several closed vesicles, Graafian follicles are formed, each of which is lined with a layer of epithelium, and contains one or two nucleated ovules, so that both stand in a definite developmental relation to the germinal epithelium. These facts may be demonstrated equally well in the ovary of the bitch; a zone of tissue exists under the germinal epithelium in which closed tubes are met with, which run in very various directions. Many of these look as if they were connected with each other so as to form a network. Deeper, there is a zone in which separate follicles exist.

Ovum and Discus Proligerus.—The ovum itself and the cells of the *Discus proligerus* may be studied in fresh ovaries. The contents of a large Graafian vesicle of the rabbit's or guineapig's ovary are discharged on to an object-glass for the purpose. They can also be well seen in the preparations above described.

Fallopian Tubes, Uterus, Vagina, and External Organs.—These may be best studied in sections of organs hardened in chromic acid—the methods recommended in Part I. being employed for the study of the several tissues of which they consist. Some special remarks are, however, necessary relating to the glands of the uterus. They can be best demonstrated in the *cornua uteri* of bitches or cats which have already borne young. The fresh organ is placed in common alcohol or dilute chromic acid, without opening it; after four or five days it is fit for making sections. Each gland consists of long blind tubes, which may be either single or divided. The glands are closely packed together. In each tube two parts may be distinguished; one of these, which may be regarded as the duct, is straight, and possesses an epithelium of slender pale cylindrical elements. The gland proper is convoluted, and consists of shorter elements. If the sections are steeped twenty-four hours in very dilute carmine, it is seen that this epithelium is much more stained than that of the duct. In the sow's uterus, and in those of the rabbit and mouse, it can be made out that the epithelium is ciliated.

The glands can be best prepared in lengths from the pregnant uterus of the sow. For the mode of preparation *see* Chapter III., p. 46. It is scarcely necessary to observe that for the study of the external organs, injected preparations are important.

Male Genital Organs.—The general structural relations of the testis and epididymis are best studied in sections of fresh organs (frog or mammalia), hardened in common alcohol; these must be stained and prepared in Dammar varnish in the usual way. Preparations with the blood-vessels and lymphatics injected must also be used. The latter are easily obtained by the method of puncture. The characteristic epithelium of the epididymis must be seen in fresh preparations in serum, as well as in sections. The structure of the *vasa deferentia*, *vesiculæ seminales*, prostate, urethra, and penis, may be all studied in the organs of the fœtus or of children, after hardening in chromic acid. The structure of the erectile tissues cannot be demonstrated without good injected preparations. [For details *see* the author's paper in Stricker's Hand-book.]

CHAPTER XII.

ORGANS OF SPECIAL SENSE.

Organ of Sight.—The epithelium, cellular elements, and the finer nerves of the cornea have been already treated of (Chapters II., III., and V). We have only to remark that, in order to observe the relation of the *cornea* to the *conjunctiva, sclerotica,* and *ligamentum pectinatum,* it is necessary to harden the bulb entire, and to make sections which shall include all these structures. The best and simplest method consists in placing the fresh bulb of a mature fœtus, a rabbit, a pig, or a calf, in one-tenth per cent. solution of chromic acid, for eight or ten days; having previously made two or three punctures in it with a lancet-shaped needle. After two or three days of immersion, the bulb may be cut in two with a razor, the crystalline lens and vitreous body removed with forceps, and the anterior half of the bulb (containing the *conjunctiva*, the *cornea,* the *iris,* and *processus ciliares,* the anterior segment of the sclerotic and choroid as well as the *ora serrata retinæ,* and *zonula Zinii*) put back in the solution. The necessary consistence having been obtained, a portion is cut off in such a way as to render it possible to make transverse sections through the above-mentioned structures. The sections may be treated in the ordinary way; and, if thin enough, they will also be useful for the purpose of studying the tissue of the sclerotic, choroid, ciliary processes, and iris, as well as of the *musculus tensor choroideæ.*

The cellular elements of the sclerotic may be further demonstrated in surface preparations as follows:—The *bulbus oculi* of a frog having been extirpated, is carefully freed from adherent connective tissue on an object-glass; the surface of the sclerotic is then thoroughly touched with lunar caustic: after a quarter of an hour small portions are cut off: these must be pencilled on their inner surface with a camel-hair brush, so as to remove any adhering pigment; the preparations being finally mounted in glycerin. Successful preparations exhibit branched clear spaces—canaliculi—on a brownish-ground, such as have been previously described.

Other portions of fresh sclerotic may, after pencilling, be treated with a half per cent. solution of chloride of gold, and employed both for vertical and horizontal sections. In the former, the violet-coloured cellular elements appear as spindle-shaped cells, lying between the bundles of connective tissue of the sclerotic; whilst, in the latter, they exhibit forms which correspond to the above-mentioned canaliculi. The sclerotic of a young rabbit may be similarly treated with the gold and silver solutions. For the study of the iris, choroid, and ciliary processes, several methods are employed besides that of making vertical sections through the hardened parts. The hexagonal, pigmented epithelium covering the inner surface of the uvea, which is considered to belong to the retina, can be removed from the fresh membrane with a scalpel or sharp needle, in small shreds: these must be spread out with needles and mounted in salt solution. Preparations of the same kind can also be obtained from bulbs which have been kept for a few weeks in Müller's fluid: they must be preserved in glycerin. The more or less branched pigment cells which are to be found in the substance of the uvea in different animals, but varying in number and distribution, may be prepared from the fresh tissue in a similar manner, but it is preferable to make thin sections of the membrane. For the investigation of the *musculus tensor choroideæ*, as well as the *sphincter pupillæ*, vertical sections of the human uvea from a bulb hardened in chromic acid, are most important; the sections must be immersed in very dilute carmine for twenty-four hours.

To demonstrate the *dilatator et sphincter pupillæ*, the iris of a small albino rabbit will serve. It must be cut out with great care, and after having been pencilled on both surfaces with a camel-hair brush, moistened with *humor aqueus*, must be immersed in half per cent. solution of chloride of gold for from thirty to forty minutes, whence it must be transferred to acidulated water. There is also another plan which answers satisfactorily:—The bulb of a similar rabbit is placed in Müller's liquid for a few days, the cornea having been previously punctured. The whole iris is then cut out, pencilled in the same fluid with a camel-hair brush on both sides, and placed in spirit for from fifteen to thirty minutes. The iris should then be coloured in dilute carmine, and portions should be mounted in glycerin. With the exception of the muscles, the blood-vessels form the most important part of the uvea. For their study, injections with gelatin, coloured by carmine or Berlin blue, should be made; albino animals being preferred. For small animals the cannula should be tied into the root of the aorta, the *aorta thoracica descendens* being ligatured. For large animals the common carotid may be employed; of course, as a general rule, only one eye will be injected. The bulb having been kept in spirit for a few days, the whole uvea is carefully isolated from the outer coats: in the case of a small rabbit, one section may be mounted including a portion of the iris, ciliary processes, and the anterior half of the choroid; and another including a portion of the posterior half of the choroid. The *circuli arteriosi iridis minor et major*, the vessels between these, the system of capillaries of the ciliary processes, and their relation to the *arteriæ ciliares posticæ*, the system (arterial) of the *laminæ Ruischii*, and the tributaries of the *venæ vorticosæ*, are severally to be studied.

The crystalline lens, with its several parts (capsule, epithelium lining the inner surface of the anterior portion, and the constituent fibres of the lens itself), should be made the subject of careful observation. The hyaline capsule, with the above-mentioned epithelium, can be demonstrated in a perfectly fresh preparation, in *humor aqueus*. The structure of the lens fibres may be made out in preparations from the lens of a fowl, or of some large mammal, macerated in very dilute sulphuric acid—(one or two per cent.). The fibres exhibit a striated appearance, and, if they are sufficiently separated from each other, it may be seen that each possesses a spherical nucleus.

In preparations of the same kind from the portion of the lens which corresponds to the margin between the anterior and posterior half of the organ, every stage of transition of the epithelium which lines the anterior part of the capsule, into true lens fibres, can easily be made out; the elements becoming progressively more and more elongated, and their nuclei more and more distant from their bases. The best way to ascertain these facts is by means of sections, which show also that, posteriorly, the lens fibres are in immediate contact with the capsule. Vertical sections display the very regular mosaic due to the cutting across of the long, hexagonal fibres. They may be made after the lens has been hardened in solution of chromic acid (one-tenth per cent.), or bichromate of potash (one-half to one per cent.). The hardening may also be effected by exposing the lens to the air, and allowing it to become almost dry: sections so obtained must be mounted in glycerin. The structure of the *corpus vitreum*, consisting as it does of a perfectly hyaline gelatinous matrix, with a few extremely pale, small spheroidal cells embedded in it, may be investigated in the fresh organ, but better in sections made after the bulb has been hardened in a one-eighth to one-half per cent. solution of chromic acid. The staining of the sections with carmine or aqueous solution of anilin will prove very useful for the demonstration of the cellular elements.

The retina presents, perhaps, a more difficult task to the histologist than any other organ; the investigation of even the simplest relations of its constituent elements requiring much time and patience. The introduction of the perosmic-acid method of preparation, however, has, within the last few years, considerably bridged over our difficulties in this respect.

The most useful preparations are those made with needles. The carefully excised fresh eye of a frog, newt, rabbit, ox, calf, or pig is divided into an anterior and posterior half. The latter is placed for from twenty-four to forty-eight hours in a one-tenth per cent. solution of perosmic acid, in the dark; thence it is transferred to distilled water for twenty-four hours. After this period small portions of the retina are snipped off and teased in a drop of nearly saturated solution of acetate of potash and mounted in the same fluid. The frog's retina in particular is extremely valuable for the study of the rods and cones with their outer and inner portions, the radial fibres, the nuclei of the outer and inner granular layers, and the nerve fibres and ganglion cells, all of which are much better seen than in retinas which have been macerated in Müller's liquid. When the object is to study the relations to each other of the different strata in the retina, either of the two following processes may be employed:—

1. The posterior half of the bulb (or, when small, the whole bulb, after two or three punctures have been made in it), is placed in a two per cent. solution of perosmic acid in the dark for twenty-four hours: it is then removed, and small, oblong pieces are cut from it with a razor (these including, of course, besides retina, corresponding portions of sclerotic and choroid), and placed in alcohol for twenty-four hours or more, until they have attained sufficient consistence for sections to be made from them after embedding. The sections should be mounted in acetate of potash as before. This method answers very well for the retina of the rabbit, calf, or pig.

2. The other plan, which must also be looked upon as a good one, is the treatment with Müller's liquid. The entire bulb of one of the above-mentioned animals is placed in this liquid, having previously been punctured at two or three points. After from three to five weeks it is taken out, and cut into an anterior and a posterior half. From the portion of retina belonging to the latter, an oblong piece is removed with fine, sharp scissors (it is generally pretty easy to do this without involving the sclerotic and choroid, since the retina has usually become more or less separated from the latter by the action of the fluid), and transferred for a few days to ordinary spirit. From this it is put into dilute carmine solution for twenty-four hours, then washed in acidulated water, and, finally, after half an hour's or an hour's immersion in absolute alcohol, is embedded in the manner previously described (Chapter VI.). The sections are transferred

in the manner there indicated from the razor to the object-glass, on which, after proper treatment, they are to be mounted in Dammar.

A skilful manipulator can obtain good results with this method. Very thin sections show, in a sufficiently clear manner, the general arrangement of the rods and cones, and their relation to the elements of the outer granular layer, that of the intermediate layer to the granules of the inner granular layer; the finely granular layer, and the relation of its fine fibrillæ to the fibrils of the inner granular layer on the one side and the processes of the ganglion cells on the other; and finally, the layer of nerve fibres. The general arrangement of the radial fibres, or, rather, bundles of radial fibres, may be also made out: each bundle, attached to the *limitans interna* by a broad basis, enters the finely granular layer, thence passing through the inner granular layer (where the bundles become ramified, and enclose nuclei), then on through the intermediate layer and outer granular layer (where again ramifications and junctions are met with) to become attached, finally, to the *limitans externa.* (*See* description of Figs. 139 and 140.)

Organ of Hearing.—The outer part of this organ, including the external ear, meatus, and Eustachian tube, should be studied in portions taken from a young human subject. To prepare the *membrana tympani* (human, or from a cat or dog), it must be exposed by the aid of saw and bone-forceps—a manipulation requiring an accurate knowledge of the topographical details of the temporal bone. This done, the membrane is excised, and either stained with silver at once, to show the epithelium of the two surfaces, or pencilled on its outer surface with a brush moistened with serum, to show the lymphatics. If the gold method is used, the epithelium is also pencilled on the outer surface, and the membrane immersed in the solution from half an hour to an hour. It must then be treated in the usual way.

The study of the membranous labyrinth, especially the canal of the cochlea and the semicircular canals—is a matter requiring an immense deal of care and practice. It should be undertaken both in fœtal and adult organs. For the examination of it in the embryo, a fœtal calf or pig from ten to fifteen centimeters long may be used. The whole cartilaginous labyrinth may be readily separated from the rest of the skull after the maceration of the latter in solution of bichromate of potash (half to one per cent.) for a week or two. After separation it is placed in spirit for a few days. A second opening (besides the already existing *fenestra rotunda*) should then be made on the side opposite to it, or, better, at a point corresponding to the top of the cochlea. The whole organ is now stuck on a needle and immersed in a warm—but of course, not hot—mixture of wax and oil, so as to fill up, at least in part, the canals which exist in the organ; this is then embedded in the ordinary way, marks being made on the mass for the purpose of indicating the exact position of the preparation. Sections are then made in succession across the axes of the several canals, and are stained in weak carmine. Such sections, being readily obtained in a perfect state in the fœtus, serve as a most valuable key to the study of the adult organ.

The fully-developed organ is best studied in the ear of a small dog, guineapig, or newborn child. From the fresh jaw of the guineapig the whole of the petrous portion of the temporal bone can readily be removed, and placed for a week or fortnight in a half to a quarter per cent. solution of chromic acid, to which a few drops of hydrochloric acid has been previously added, the liquid being changed once or twice during that time. The cochlea is then removed, and after remaining in spirit for a few days, is filled with a mixture of wax and oil under the air-pump. Sections are prepared as before, after embedding. A second mode should also be employed, which is as follows:—A horizontal section is made through the organ after removal from the spirit, so as to expose all the turns of the cochlear canal. Both halves are then embedded in gelatin solution, to which a few drops of glycerin has been added, as mentioned in Chapter VI. The transparency of the gelatin enables us to be sure of the direction of our sections. These are placed first in warm water, to remove the gelatin. They may be then mounted in glycerin, or replaced for a short time in spirit, stained with carmine, and mounted in Dammar. I would, however, advise the student not to risk the manipulation required for the latter process, but to mount in glycerin at once after the warm water; for the section, if it is as thin as it should be, would stand a considerable chance of injury.

For the study of the *organ of Corti*, thin vertical parts of sections must be sought for in which the *lamina spiralis* near that organ is seen to be cut exactly across: this is more particularly the case when the situation of the rods of the arch of Corti, the arrangement of the cells of Deiter and the ciliated cells, and the distribution of the nerves of the *membrana basilaris*, are under examination. To shew the elements of the *membrana reticularis*, and the epithelium of Reissner's membrane, more obliquely cut parts of the section are to be chosen, or even portions where a surface view of these structures is obtainable.

Organ of Taste.—For the study of the organ of taste the tongue of the frog or rabbit may be used. In the former, our attention may be confined to the *papillæ fungiformes*, the most important subject of observation being the topographical relations of their cellular covering. The perfectly fresh organ is spread out with pins on a plate of cork, care being taken to avoid unequal stretching, and placed in very dilute chromic acid. Vertical sections are then made in the usual way. Another way is to colour the fresh organ, spread out on cork as above, in chloride of gold. Half an hour's steeping in half per cent. solution is sufficient; but it is necessary, before exposing the preparation in water, to stream it thoroughly with the same liquid, in order to avoid the subsequent formation of coloured deposit on the surface. As soon as the tongue has assumed the proper colour, it must be hardened in alcohol, for the preparation of sections which must be prepared in glycerin.

In vertical sections of fungiform papillæ the following parts are seen:—In the axis of the papilla, along with the vessels, a nerve twig is observed, consisting of medullated fibres, which ascends towards the summit of the papilla, and there pencils out into nerve fibres. Each of these is seen eventually to end in a non-medullated fibre. Along the border of each papilla are seen muscular fibres which divide dendritically as they ascend. The covering of the flattened summit consists of a relatively thick layer, in which two strata can be distinguished. The more superficial of these is thicker and paler, and is finely striated in the direction of the long axis of the papilla. In thin sections it can be recognized that this material consists of pale longitudinally striated cylinders. The deeper and thinner stratum consists of a ground-substance deeply stained both by gold and carmine, in which several layers of nucleus-like structures are embedded. It can be made out in very thin sections (and also in teased preparations) that the cylindrical nucleated cells take part in the formation of both layers, the outer segment of each cell contributing to form the outer stratum, the other, which contains the nucleus, the inner stratum. The outer segment of each cell is pale and finely streaked longitudinally, while the inner segment, which consists of granular protoplasm, is divided towards the papilla into branched processes, which unite with each other and with those of neighbouring cells. In preparations successfully stained with gold, it can further be made out, that the non-medullated fibres resolve themselves into a network of extremely fine fibrils, which spread under the stratum of cells. No connection, however, has been demonstrated to exist between this network and the anastomosing branched processes above mentioned. The forms of the cylindrical cells should be also studied in teased preparations. Strips of fresh mucous membrane are placed in the dark for from twenty-four to forty-eight hours, in one-tenth per cent. solution of perosmic acid. The object having been steeped in water one or two days, shreds must be torn off the free surface of each strip of membrane, with fine sharp needles. Each of these shreds having further been teased carefully with needles, must then be mounted in a drop of acetate of potash. Another method consists in macerating similar strips in iodized serum, solution of bichromate of potash, or very dilute solution of chromic acid (one-twentieth per cent.). The teased preparations must be mounted in glycerin.

At the edge which unites the dorsal and lateral surfaces of the tongue of the rabbit, a round or oval depression is seen, on the surface of which an arrangement of furrows with intermediate ridges are visible to the naked eye. If a vertical section is made of this part, in a tongue hardened in one-tenth per cent. chromic acid, in such a direction that the plane of section crosses the ridges, a meshwork of trabeculæ of striped muscular fibres, in the spaces of which the numerous mucous glands are embedded, can be recognized. The short ducts of these glands rise for the most part vertically, but occasionally obliquely to the surface; always opening into the splits between the ridges. So much of the mucosa as lies underneath the furrows and ridges, contains a great number of non-medullated nerve-fibres. Each ridge is covered with a layer of epithelium which becomes thicker upwards, *i.e.*, towards the *arête;* and on either aspect of each ridge, certain bodies are seen, embedded in the surface by which it looks towards its neighbour: to these the term *taste goblets* (*Geschmacksbecher*) has been applied. They are, as the term indicates, bell or cup-shaped structures, which are limited by a special layer of flattened epithelium cells, which in profile look spindle-shaped. Into the space enclosed within this layer, there projects from the mucosa a bunch of oblong spindle-shaped cells, which, towards their bases, appear to be divided. Each contains an oblong nucleus. The forms of the elements just described, and of those which constitute the outer wall or investment of each goblet, should be studied in teased preparations.—The circumvallate papillæ of the human tongue and of other mammalia exhibit similar structures.

Organ of Smell.—Teased preparations can be obtained by macerating the olfactory mucous membrane of the frog or of mammalia in one-twentieth per cent. chromic acid, in Müller's liquid, or iodized serum, or perosmic acid. The whole of the head of the frog, after removing the lower jaw, and opening the nares, is placed in the liquid. In mammalia, the nares can be opened in the middle line, after which portions of the olfactory tract can be removed. For the preparation of sections, the parts must be kept in one-fifth per cent. solution of chromic acid, which must be renewed as often as necessary till the bone becomes soft. In teased preparations it is seen that there is no marked distinction between the ordinary conical epithelial cells and the special spindle-shaped cells, recognized as olfactory epithelium; for they are connected together by a continuous series of transitional forms. The most characteristic form of the olfactory cells is drawn out at both ends, viz., towards the *mucosa* into an extremely slender filament, which exhibits granular swellings; and towards the surface into a somewhat stouter fibre, which is streaked longitudinally, like the ordinary epithelial element, and, like it, bears at its extremity a bunch of cilia; but, as has been already said, examples are met with, in which the special peculiarities are wanting. In the frog, the processes of the epithelial elements appear to penetrate the mucosa, so as to form a network of fine trabeculæ. The finest branches of the olfactory nerve are seen to tend towards this network, but have not been traced into actual continuity with the extremities of the so-called olfactory cells.

The mucosa and its glands must be studied in sections.

CHAPTER XIII.

EMBRYOLOGY.

In treating of the methods which are commonly employed in the study of general Embryology, we shall follow the same plan as in special Histology; noticing only those points which are of importance to the beginner.

As is well known, three parts are distinguished in every mature egg: the vitelline membrane, the yolk or vitellus, and the germ. The last-mentioned is the essential part, and assimilates itself to the general idea of the cell, viz., an organism composed of protoplasm, which possesses the capability, under certain conditions, of performing amœboid movements. In the protoplasm of the germ the germinal vesicle, a body analogous to the nucleus of other cells, is embedded; and within this lies the germinal spot, the analogue of the nucleolus. According as the two elements of the egg, which are enclosed by the vitelline membrane, viz., germ and yolk, exist separately from one another, or form a single body, eggs are subdivided into two large groups, viz.:—meroblastic eggs, in which the germ is separate from the yolk—such as those of the bony fishes, scaly reptiles, and birds; and holoblastic eggs, in which the germ itself contains the elements of the yolk—those of the cartilaginous fishes, amphibia, and mammals.

In eggs of the first group, the germ lies upon the yolk in the form of a disk; for which reason it receives the name of Blastoderm: formerly it used also to be termed (after Reichert) "formative yolk," while the yolk itself was called "nutritive yolk." The first process that claims the attention of the embryologist is cleavage. The fertilization of the egg sets this process going. It is called cleavage because the germ divides into two cleavage masses, each of these again into two, and so on; until the whole germ is divided into a number of globules, each of which consists of protoplasm enclosing a vesicular nucleus, and, like the entire germ, is endowed with the capability of performing amœboid movements. These cleavage globules are called "Embryo cells." Only the germ or blastoderm takes part in the cleavage, since this alone is endowed with amœboid movement. Consequently in meroblastic eggs the cleavage is said to be partial. In the holoblastic, on the other hand, the whole egg divides, for the whole is germ; it is, therefore, said to exhibit total cleavage.

Study of the Process of Cleavage in the Ova of Fish and Amphibia.—The cleavage process should be studied, in the first place, in the entire ovum; the knowledge thus gained being completed by sections of the germ at the cleavage time. Of meroblastic eggs, those of the trout are best suited for this study. Several such eggs are examined under the microscope in a watch-glass, in the water in which they have lain since undergoing fertilization, strong transmitted light and a weak magnifying power (90–100) being employed (*see* figs. 159–168). At the tenth hour after fertilization, the blastoderm appears, lying upon the yolk like a lid over a saucer-shaped depression; the yolk which forms the bottom of this cavity contains closely packed oil globules, which have become aggregated at this pole of the yolk since the time of fertilization. In the blastoderm amœboid movements are observable. About the twelfth hour, the first cleavage line appears. About the twenty-seventh, almost all the eggs show two cleavage lines crossing each other. Between this time and the end of the second day, eight segments may be distinguished; so that four cleavage lines are now seen on the surface of the blastoderm. At the end of the seventh day the process of cleavage has progressed so far that the surface of the blastoderm appears beset with a number of bosses, like a mulberry. The cleavage process is far more easily studied in the holoblastic eggs of amphibia. If eggs of the frog or toad, freshly spawned, are placed under the microscope, in a small cell, which may be conveniently prepared upon a slide by means of putty, it is seen—(especially in the case of the latter, where they are placed one behind the other in rows in gelatinous strings),—that only a very few are spherical: generally one part of the surface is flattened: so that it frequently happens that, in a long row of eggs, alternating conical ones are met with. About the sixth or seventh hour after spawning, it can be seen by transmitted light that most of the eggs have become round. As this period of time approaches, the amœboid movement of the germ becomes more distinctly visible, presenting the appearance of an oscillation at some point or other within the vitelline membrane. This appearance gradually increases, until a slight indentation like a notch is seen at some part of the margin by transmitted light. This first notch fills up, but soon a similar notch occurs in another spot, which is permanent. By strong reflected light, if the egg lies in such a position that the white pole is directed downwards, a crater-like dimple may be seen on the surface. This dimple extends itself over the margin of the hemisphere, diminishing at the same time gradually in depth. It is called the plaited band (*Faltenkranz*), because a number of smaller creases proceed from it at right angles. This appearance owes its name to the erroneous impression that it is due to a folding of the vitelline membrane, but in reality it merely depends on the amœboid movement of the germ. In fact, it is possible, by close observation, to convince one's self that the furrows of the plaited band are subject to active changes, for successive groups of them disappear, again crop up, become more extensive and deeper, and then again retire. After a longer or shorter time—commonly one hour from the appearance of the first dimple—one of the folds of the plaited circle becomes deeper, and spreads itself more and more towards the periphery of the hemisphere, whilst the others gradually disappear. Eventually a deep cruciform furrow is apparent in the hemisphere we have hitherto had under observation, and which, as previously stated, is on the opposite side to the white pole. We will call this the *upper* hemisphere. At this time, only a single shallow furrow is seen in the *lower* hemisphere. Subsequently the furrowing proceeds somewhat more rapidly; for the third, or *equatorial* furrow, occurs half an hour after; other furrows then appear at right angles to the three first formed, generally in the same succession in which the principal furrows have originated; from these secondary furrows of the first order proceed others of the second, and from these, others of the third, and so on. The upper hemisphere divides much more quickly than the lower.

The ova of the trout are prepared as follows:—The egg is placed upon an object-glass between the points of a broad pair of forceps, so that the blastoderm is uppermost; the forceps are held with their blades at a fixed distance from each other, while the egg is pierced near its equator with a lance-shaped knife. On rapidly withdrawing the knife, it generally happens that the blastoderm *in toto*, with a large part of the tenacious semi-fluid yolk, spirts out. The object must now be surrounded with a ring of putty and covered. The attention of the observer should be directed to the appearance of the elements, their amœboid movement, and to the various forms of cleavage. The preparation of the ova of Batrachia is far simpler. The egg is placed upon an object-glass, and as much as possible of the gelatinous investment is removed with the aid of forceps and scissors. The vitelline membrane is ruptured by means of needles, and a small portion of the escaping contents is spread out in a very thin layer. If the egg is not more than three days old, it can be investigated under low powers (Hartnack's 5 or 7) without a cover-glass. The yolk disks should be especially observed, and the active movements of the pigment granules with which the embryo cells are filled. Attention should be further directed to the hyaline prominences which the latter send out and retract, particularly after the addition of a very small drop of distilled water.

The Cleavage Cavity.—The second important point, to which the embryologist should direct his attention, is the cleavage cavity. In the trout, this comes into existence towards the end of the cleavage process. The blastoderm appears to be separated from the yolk of the saucer-shaped depression by a cavity which gradually increases in width and depth. The blastoderm is not, however, entirely detached from the yolk, but remains connected with it here and there by chains of cells. These chains of cells—"sub-germinal processes"—may be compared to columns by means of which the blastoderm rests upon the yolk (*see* fig. 167). The cells of the subgerminal processes, like those of the deeper layer of the blastoderm, are larger and more coarsely granular than those of the more superficial layers. By degrees the cells of the subgerminal processes become separated from the blastoderm, and lie upon the floor of the cleavage cavity. The elements which are found in this position are characterized by their greater size, and by their distinctly granular appearance; they are products of the blastoderm, which are either left lying on the floor of the cavity when it is formed by the raising of the blastoderm from the yolk, or fall to the bottom of the cavity as it increases in size.

For the study of the formation of the cavity, that is, of the elements which are to be found on its floor (the destination of which we shall again have occasion to mention) and of the simultaneous expansion of the blastoderm over the cavity, sections are alone available. Eggs of the requisite stage (10–14 days) are placed in a very dilute (one-tenth per cent.) solution of chromic acid, the liquid being frequently changed. After a few days the eggs will have become almost black and quite friable. An egg is now pierced with a lance-shaped needle, and the vitelline membrane carefully torn open at one place by means of sharp forceps, the rent being extended in a horizontal direction until it describes a complete circle; the membrane is then removed from the upper hemisphere, which contains the blastoderm. Thereupon the blastoderm, together with the whole of the yolk of the saucer-shaped depression, is separated by a sharp scalpel and placed in dilute alcohol, where it may remain for any length of time. It is, however, ready for further treatment in one or two hours. It may be stained by steeping it for twenty-four hours in very dilute carmine (*see* Chapter VII.), and it is then washed in weakly acidulated water. The object is now placed in absolute alcohol for-from half an hour to an hour. After this, it is embedded in the following manner:—A layer of the mass used for embedding (wax and oil), is poured upon a flat piece of glass, wood, or cork, or into a little box, and is allowed to harden; the object, after its surface has been carefully dried, is placed in the desired position upon this mass, and a further layer is poured around and over it, which must be warm, but not too hot. When the mass is thoroughly solidified, sections are made as follows:—The razor is moistened, by means of a small brush, with oil of cloves or with turpentine, and a section made, which is floated off from the razor to an object-glass with oil of cloves. When the section is thoroughly transparent, a process which occupies a few seconds, or at most minutes, if the object has been long enough in absolute alcohol before embedding, the excess of oil of cloves is to be carefully soaked up with strips of filter-paper. A window is cut out of fine tissue paper, and applied to the preparation in such a way as to afford protection from the pressure of the cover-glass. A drop of Dammar varnish is allowed to fall upon the preparation thus enclosed by the paper, and the whole is covered. The eggs having been placed in one-tenth per cent. solution of chromic acid until the gelatinous investment is entirely dissolved, they are transferred to common alcohol for two or three days and then preserved in glycerin. They may be used even after an interval of months.

For the study of the cleavage-cavity of Batrachia, sections should be made of the eggs of Bufo, beginning with the stage at which the first furrows are already formed. The egg is taken, by means of a spoon, out of the glycerin, dried with filter-paper, and embedded according to the method above described. The razor in this case is to be moistened with absolute alcohol, and the sections floated on to the object-glass, with the same liquid. The alcohol is removed by filter-paper, and the section moistened with a drop of oil of cloves, after which the process is the same as above. Batrachian eggs require great care and attention, both in making and handling the sections; first, because the ovum is less easily fixed than is the case with the disk-like germ of the trout or chick, and, further, because it is extremely friable, so that sometimes, out of ten sections, only one will be brought entire under the cover-glass. The first indication of a cavity may be traced shortly after the appearance of the first two furrows. In sections made at this stage, it is seen that the upper two quarters of the germ, that is to say, those furthest removed from the white pole, and which are always smaller than the two lower, are rounded off at their inner angles, *i.e.*, those turned towards the centre of the germ, as if they had retracted from it; the lower two, also, are somewhat rounded at their inner angles, but not so markedly as those above: by this means a small cavity is formed, which lies just in the place where the four segments meet. In sections of progressively later stages, it will be observed, in the first place, that the upper segments have undergone cleavage much more rapidly—in other words, that their elements are considerably smaller; and, secondly, that the cavity becomes enlarged at the expense of the upper half of the germ. In a still later stage of cleavage, forms will be met with in which the cavity takes up the greater part of the space occupied by the upper segments. The cavity is spanned by a thin dome, consisting of only two or three layers of small elements; whilst its floor is flat and lined by larger elements belonging to the lower segments. Underneath these elements, which still contain pigment, elements occur which become larger as the white pole is approached. At this time it may be observed, that these large elements—which may be termed "formative elements"—spread upwards from the floor of the cavity over the under surface of the dome, until at last a stage is reached at which the whole of that surface is covered with them. In the middle part of the dome these formative elements are disposed in a single layer; on the parts which are in closer proximity to the floor of the cavity, the number of layers is greater. The dome consists, therefore, at this stage, in the first place, of two or, at most, three layers of small elements which originally belonged to it (and which are also continuous with the cortex of the rest of the germ); and secondly, below these, in its central part, of a layer of larger elements, which before formed part of the floor of the cavity.

Simultaneously with the changes just mentioned, another important change occurs at the white pole, as may be ascertained by the study of sections at different stages. This pole has been getting gradually smaller, and now presents the appearance of a sharply bounded white patch of the size of a pin's head—the so-called yolk-plug (*Dotterpfropf*). A fissure occurs, which constantly extends further and further upwards, increasing at the same time in width, until it gradually expands to a cavity, which is eventually only separated from the cleavage-cavity by a single layer of the larger elements. As this cavity (called the visceral cavity, Rusconi's cavity, *Leibeshöhle*) increases, the cleavage-cavity diminishes. In consequence of these changes, the position of the egg is altered; that which before was the upper half now becoming the lower. (As regards the formation of the cleavage and visceral-cavities, compare figs. 169–173.)

Formation of the Lamellæ of the Blastoderm.—From a comparative study of sections of the egg of the trout at successive stages, from that at which the blastoderm begins to form a cover over the saucer-shaped depression, consisting of a middle thinner, and a peripheral thicker part (marginal swelling—*Randwulst*), to that at which it has already grown round a quarter of the yolk and exhibits the first trace of the formation of an embryo, the following facts may be made out:—The large elements found on the floor of the cavity gradually tend towards the periphery of the blastoderm, where they form the peripheral thickening, or marginal swelling already mentioned (*see* fig. 168). As this occurs the central part of the blastoderm by degrees becomes so thin, that it consists at length of only two layers of cells, an upper lamella of flattened elements, and a lower containing loosely arranged spherical elements (in single or, here and there, in double series). These two layers are continuous with the marginal swelling, the upper layer of which also consists of flattened elements, the lower of one or two strata of more or less cylindrical cells. In the marginal swelling two other strata exist underneath these layers, each of which consists of large spherical elements, and is at least two cells deep. We have therefore in the marginal swelling, by the thickening of which the rudiment of the embryo is formed, four layers, the upper or corneal layer (*Hornblatt*); a second, or, as it may be termed, nervous stratum, because out of it is formed the central nervous system; a third or motor-germinative; and a fourth, or epithelial glandular layer (*Darmdrüsenblatt*). Of these four layers the two lower must be attributed to the formative elements which come from the floor of the cavity.

To the conditions just described those found in the batrachian egg are analogous. The mode in which, during the formation of the cleavage-cavity, formative elements spread from its floor over the under surface of the dome, adding a third stratum to the two of which it already consists, has been already described. This third layer then splits into two, whilst the visceral cavity is growing upwards into the dome. At a point which corresponds to the central part of the cavity the cortex becomes thicker: this thickening, which is formed at the cost of the second layer, is the rudiment of the central nervous system of the embryo. We find the same four layers in the egg of Batrachia—the corneal, the nervous, the motor-germinative, and the epithelial glandular (*Darmdrüsenblatt*): the last two of which, as in the ovum of the trout, are derived from the formative elements of the floor of the cleavage-cavity (*see* fig. 173).

Cleavage Cavity of the Chick.—For the study of the cleavage process and formation of the cleavage-cavity, in the blastoderm of the chick, it is necessary to intercept the eggs in their passage through the Fallopian tube; for in eggs which are already laid, these processes have been gone through. The investigation of these phenomena is expensive, and depends somewhat on chance. Hens known to be in the habit of laying eggs in spring and summer must be sacrificed. Eggs may be examined in which the shell is either absent or consists of a very thin parchment-like structure, or is in process of calcification. They are placed for a few days in a deep capsule containing a one per cent. solution of bichromate of potash, and are hence removed to a one-sixth per cent. chromic acid solution for one or two days. After this time the part of the yolk which has the blastoderm resting on it, is cut off with a razor and laid in common alcohol, in which with due precaution the vitelline membrane can be readily stripped off from the blastoderm. The subsequent processes are the same as with the blastoderm of the trout.

If eggs in the different stages of their passage through the Fallopian tube have been obtained, it is easy to make out in prepared sections, that, during the formation of the cleavage-cavity, the large coarsely granular elements (filled with the coarse granules of the yolk), which compose the deeper layers of the blastoderm, remain lying in large numbers upon the floor of the cleavage-cavity; that these are most numerous towards the *area opaca*, that is, where the peripheral part of the blastoderm lies upon the white yolk (yolk-rim, *Keimwall*); and that they here become continuous with the large coarsely granular elements of the deeper layers of the blastoderm. These elements lying on the floor of the cavity and derived from the blastoderm during the formation of the cavity, correspond to the formative elements on the floor of the cleavage-cavity of the trout's egg, and those elements which, in the batrachian egg, stretch up from the floor of the cavity to the under surface of the dome (*see* fig. 175).

Lamellæ of the Blastoderm of the Chick.—The study of the layers of the embryo of the chick must be commenced with fresh laid eggs. The egg is held with its long axis horizontal; the shell is cracked at its upper pole; the bits of shell in this place are removed with a forceps, and the outer membrane torn off the exposed part; the shell is then broken in two, and the contents are let out into a flat capsule. With the aid of scissors and forceps, the egg (using the word in its more restricted sense) is freed from the investing albumen, which is carefully poured off. After having, by means of a lens, acquired a general notion of the grosser anatomical relations as they present themselves on a surface view (such as the *Area pellucida, A. opaca,* Pander's " nucleus of the white yolk," &c.), we pour into the capsule in which the egg lies a small quantity of one per cent. solution of bichromate of potash, which, after one or two days, is replaced by from one-sixth to one-tenth per cent. chromic acid solution. In two or three days more, the segment of yolk which bears the blastoderm is cut off and transferred to spirit; the vitelline membrane is then carefully removed. Afterwards the object, which may or may not be stained, is treated with absolute alcohol, embedded, and employed for sections in the manner above described. This method may be employed during the first twenty-four hours of incubation. At a later period, or at all events after thirty-six hours, the egg must be treated in the following manner:—

After the yolk is freed from albumen, the vitelline membrane is snipped with scissors at a point in its periphery as far removed from the blastoderm as possible; part of the yolk flows out through the opening, while the blastoderm adhering to the vitelline membrane remains in position. The vitelline membrane is then cut around the blastoderm, the circular piece not only including the blastoderm, but the vitelline membrane over it, together with a portion of yolk under it. This is placed in a small flat watch-glass, which is held by forceps, and is brought into a glass capsule containing a very weak solution of bichromate of potash. After from ten to fifteen minutes, the edge of the vitelline membrane is seized by forceps, and gently swayed to and fro in the liquid till that membrane is loosened and removed. The blastoderm, with the yolk adhering to its *area pellucida*, is thus completely isolated.

In the superficial portion of the germ disks thus isolated, especially those of the early part of the second day of incubation (provided that they are normally developed as is usually the case in spring and summer), the primitive streak, the rudiments of the central nervous system, of the chorda dorsalis, of the protovertebræ, of the heart and great vessels, of the eyes, of the auditory vesicles, and of the olfactory pits, may be observed. For this purpose, the blastoderm is floated from the watch-glass on to an object-glass, and examined with a low power. For studying the first vessels it is necessary to use higher powers. The object, in solution of bichromate of potash, or in a mixture of this and glycerin, should be surrounded by a ring of zinc foil, wax mass, putty, or sealing-wax, and covered. The whole germ disk of the second day of incubation, which is very suitable for the demonstration superficially of the rudiments of the organs just named, may be preserved for a considerable time, if the wall of sealing-wax surrounding the blastoderm is high enough. The mixture consists of one part of one-sixth per cent. chromic acid, two parts of one-half per cent. bichromate of potash, and one part glycerin. The cover-glass is fixed by means of sealing-wax.

Sections through the unincubated germ-disk show that it consists of two layers, besides the formative elements which are to be found on the floor of the cleavage-cavity, and at the yolk-rim. (*See* fig. 176.) Sections made during the first half of the first day teach that these formative elements find their way from the yolk-rim in between the two layers of the germ, so as to form, first (seventeenth hour), the central part of the middle layer of the *area pellucida*, and afterwards (at the twenty-third or twenty-fourth hour), the remaining portion of that layer. Thus, at the end of the first day, the germ-disk, which before consisted of two layers, consists in the *area pellucida* of three—upper, lower, and middle—the last originating from the formative elements which had previously rested on the floor of the cleavage-cavity.*

As the central nervous system is developed from the central portion of the upper layer, the remainder of this layer giving rise to the epithelium of the skin and of the cutaneous glands, it follows that the upper layer in the chick represents the upper and nervous layers in fish and Batrachia; it is therefore simply called *corneal* layer: the middle layer in the chick corresponds to the third in the trout and in Batrachia, and is therefore termed motor-germinative; the lowest layer in the chick corresponds to the fourth in the germ of trout and Batrachia, and is termed the epithelial glandular layer.

When the central part of the middle germinal layer is formed (seventeenth hour), the upper one is seen to be thickened at its middle portion; it consists of cylindrical cells. At the same time, this middle portion of the upper layer is more or less fused with the just deposited central part of the middle layer. This condition shows on a surface view the primitive streak (*Axenstrang*). Along with the formation of the primitive streak, the dorsal groove is also developed, a differentiation of the middle layer of the germ takes place into the notochord and protovertrebræ, and the dorsal laminæ begin to project. In the first hours of the second day of incubation, the dorsal laminæ are seen to be already approaching one another, so that in the region of the neck they almost touch; at the tail end they are still a considerable distance apart, so that the dorsal furrow is very shallow. A short time afterwards, the dorsal laminæ in the cervical region are observed to be completely closed, and the dorsal furrow is changed into a canal—the central canal of the central nervous system. (Figs. 177, 178.)

In sections made later in the second day of incubation, the rudiments of the notochord and of the protovertebræ appear in the central part of the middle layer of the germ; the two outer portions of this same layer—the ventral laminæ (*Seitenplatten*)—split into an upper parietal (*Hautmuskelplatte*) and a lower visceral lamella (*Darmfaserplatte*); between the cleft or split thus formed is the rudiment of the pleuro-peritoneal cavity. (Figs. 180–182.)

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* The corneal, motor-germinative and epithelial glandular layers, correspond to the epiblast, mesoblast and hypoblast of Huxley.—Ed.

At the same time, the rudiment of the Wollfian duct appears on the upper surface of the middle layer of the germ, where the rudiments of the protovertebræ abut on the ventral laminæ. In sections through the blastoderm made during the second day (36–48 hours), the protrusion of the primary optic vesicles out of the anterior cerebral vesicles may be studied as well as the intrusion of the secondary eye vesicle into the primary (*see* fig. 185 *b*), which proceeds simultaneously with the formation of the rudiment of the lens by the thickening and subsequent separation by constriction of the intruded part of the corneal layer. Similarly the auditory vesicle presents itself as a pit-like depression of the same layer; this pit gradually deepens whilst the margins rise up and grow until they fuse into one another, so as to form the auditory vesicles. We may further notice the extrusion of the visceral lamella in the region of the neck, which forms the wall of the heart vesicle. Sections made on the second and the commencement of the third day serve for the study of the development of the amnion, as a fold-like elevation of the corneal and parietal layers, as well as that of the intestinal groove, and of the *fovea cardiaca* (*Vorderdarm*) by the closing in of the epithelial glandular layer (*see* fig. 181). The extrusion of the two primary hepatic ducts out of the tube so formed, its partition into a posterior œsophageal and an anterior tracheal-tube, and the extrusion of the lungs from the latter must be followed at later stages of incubation.

CHAPTER XIV.

(APPENDIX.)

STUDY OF INFLAMED TISSUES.

Inflammation of Epithelium.—The inflammatory changes of the epithelial elements of the cornea may be studied by abrading the epithelium over a limited surface in several frogs, and examining the organ at various periods after the injury. The cornea must be studied in the fresh state (with and without irrigation with serum), as well as after preparation with gold and hardening in alcohol. Sections in both directions must be made of the preparations so obtained. Evidence is thus obtained (1) of the division of the nuclei of the epithelial cells, (2) of the overgrowth of the bodies of the cells, and (3) of their subsequent division.

The examination of the catarrhal secretions of any inflamed mucous membrane which is covered with pavement epithelium, is very instructive. If a small drop taken from the surface of such a membrane is examined, either without any addition, or diluted with a drop of serum, it is seen that among a great number of amœboid young cells (pus cells) a few larger structures are to be found, consisting of granular protoplasm, which, as regards their form and size, and the characters of their nuclei, resemble epithelial cells. Some of them contain vacuoles of very various size, each exhibiting in its wall a well-defined nucleus, which either shows constrictions or is already divided. In those vacuoles which are largest there are pus-corpuscles. Besides these, thin-walled vesicular bodies are seen, of great size, filled with pus-corpuscles; and between them and the cells containing vacuoles there are all transitions. If vertical sections are made of a bit of the inflamed mucous membrane after treatment with gold, it is learnt that these structures correspond to the cells of the superficial layers. In fresh preparations taken in such a way as to include the elements of deeper layers, large epithelial cells are seen which exhibit very distinct indications of division both in their bodies and nuclei. On the warm stage these cells may be seen actually dividing. To obtain permanent preparations, the fresh inflamed mucous membrane must be placed in two per cent. solution of bichromate of potash. After two or three days, sections may be made by shaving off a portion of the mucous membrane, and comminuting it in a drop of glycerin with a blunt instrument. It need scarcely be added, that both those cells of the deeper layers which are in the natural state, and those which exhibit appearances of division, have the ridged character. Similar changes can be studied in certain chronic diseases of the skin, as in acuminated condylomata. (*See* Chap. II.)

Inflammation of Endothelium.—As regards the endothelium of the serous membrane, the changes consequent on inflammation have been already referred to. In the blood-vessels, the inflammatory changes may be studied by cauterizing the external surface of any superficial vein (*e.g.*, the external jugular or femoral), or even by simply ligaturing the vessel. Three or four days after the injury, the vessel is excised and hardened in chromic acid, or treated with gold and hardened in alcohol, for the preparation of sections. When the vessel is very thin-walled, it can be studied at once, without preparation, after staining with gold or silver. The appearances correspond to those observed in the serous membranes.

Inflammation of Cartilage.—Germination of the cells of hyaline cartilage can be studied after mechanical injury of articular cartilages. The best method is to pass a needle into the knee-joint of a rabbit in such a way that it penetrates into the tibia. A few days after, sections are made of the fresh cartilage, and stained in gold. It is more difficult to observe inflammatory changes of the cartilage cells in the frog. Much can be learnt from cartilages of human joints in a state of chronic inflammation.

Inflammation of Bone.—Germination of the cells of bone may be induced in the long bones of mammalia by passing a red-hot needle as deeply as possible into a bone, previously freed of the soft parts covering it, and then cauterizing the hole with a pointed stick of nitrate of silver, or by violent fracture. After a week or more the bone is excised. Scale-like bits are then split off from the immediate neighbourhood of the injury, and steeped in chloride of gold, and then placed in water acidulated with acetic acid till they are soft enough to render it possible to make sections, which must be prepared in glycerin. Another plan is to place the part in solution of chromic acid ($\frac{1}{8}$ to $\frac{1}{4}$ per cent.), to which hydrochloric acid has been added, as described fully in Chap. II. The sections should be so made as to comprise the transition between inflamed and normal conditions. Human inflamed bones can often be studied in amputated limbs. In all of these cases the lacunæ are seen to contain groups of young cells, instead of the ordinary branched cells.

Inflammatory Changes in the Liver Cells.—Inflammation of the tissue of the liver may be induced by passing a needle into the organ. Twenty-four to forty-eight hours after the injury, the animal must be killed. The liver cells exhibit distinct appearances of division and germination. Similar appearances are seen in the neighbourhood of the so-called psorosperm nodules in the liver of the rabbit.

Inflammation of the Cornea.—Inflammation of the cornea may be studied in the frog in two ways:—The cornea may be cauterized at the centre, to such a depth as almost to perforate it, or a thread may be drawn through it entering at the centre and passing out through the sclerotic, beyond the margin, the ends of which are then tied. After cauterization it is necessary to wash the part with a few drops of solution of common salt. In either case the animal is placed in a beaker glass, with some moist blotting-paper at the bottom of it. To study the successive stages of the process, half-a-dozen corneas should be prepared in this way at a time, which can then be excised after 8, 12, 18, 24, 36 and 48 hours. The best preparations are obtained from *rana esculenta*, during the summer months, from 8 to 24 hours after the introduction of a silk thread as above described. The cornea should be studied first in the fresh state, and then stained with gold. It is excised in the manner directed in Chapter II. and prepared in humor aqueus, care being taken to protect it from pressure by inserting slips of fine paper under the edges of the cover-glass. The contrast between a cornea twelve hours after injury and a normal one lies, first, in the immense number of migrating cells it contains, and secondly, in the marked distinctness of the branched corpuscles. The migrating cells are most numerous towards the periphery, occurring more and more scantily towards the centre. They are masses of protoplasm of irregular form, beset with knob-like prominences, and exhibit very active amœboid movement. To study their changes, the preparation must be irrigated with serum. For this purpose, a frog is decapitated and the blood received in a porcelain capsule and allowed to coagulate. The serum is collected in capillary glass tubes. The irrigation is performed as before directed (Chapter I.), a very small strip of blotting-paper being used. Under the immersion objective, the most active motions can then be observed; and if a single corpuscle is kept under observation for a length of time, it is sometimes possible to make out an appearance as if it were about to divide. A line presents itself on the surface, which after a time assumes the character of a furrow. Occasionally the furrow is seen to deepen till the two parts are severed. In other cases, one of the knob-like prominences enlarges and separates itself. As regards the branched cells, some of them appear to be larger than natural, while their processes become thicker and less branched. Immediately under the epithelium, as well as under the endothelium of the posterior surface, the processes often exhibit node-like enlargements. Occasionally corpuscles occur which possess processes only on one side, while on the other they merely exhibit slight prominences. If a cornea of this kind is immersed in solution of chloride of gold for twenty minutes and treated as usual, the corpuscles are seen to be much more stained in certain parts than in normal corneas, although the latter may have been immersed twice as long. If a comparison is made between different parts, it is easy to satisfy oneself, that the strongly coloured corpuscles are larger and look as if they were swollen, and that their processes are few in number and thicker. The nuclei of these corpuscles exhibit the most various phases; constrictions and bulgings are seen in some, complete division in others.

This is by no means the final stage of the alteration of the corpuscles. It may be demonstrated at a later period that in some parts no branched corpuscles can be distinguished, their place being taken by a trellis-work of spindle-shaped cells, presenting the aspect of parallel streaks of granular protoplasm, running in two directions at right angles to each other. In each streak there are thickenings at intervals. Each thickening may contain either a few deeply stained small nuclei, resembling those of the neighbouring migratory cells, or nuclei with constrictions which resemble those which are characteristic of the cornea corpuscles. Between these larger swellings containing nuclei, the streaks are beset with small nodosities of various sizes. If these streaky parts are compared with others, it is seen that there are all transitions between the streaks and regularly branched oblong cornea corpuscles, while in other directions their relation can be traced with chains of young cells, which run in the same directions as the streaks.

The entrance of migratory cells, and even a beginning of the changes above described in the cornea corpuscles, may be imitated in an excised healthy cornea, as follows:—Inflammation is produced in one eye by cauterization, and then, twenty-four hours after, a portion of the cornea of the other eye is excised, spread out carefully, and lodged between the membrana nictitans and the cornea of the injured eye. The membrana nictitans is then drawn up and secured by two or three ligatures to the skin. After twenty-four hours more, the sac is opened and the cornea taken out. It may be examined in the fresh state, and after preparation with gold.

Corneas prepared in other ways (*e.g.*, by gentle friction with solid caustic, as directed in Chapter II., or by holding the head over hot water, and brushing the surface with a camel hair pencil) and then excised and stained in silver solution, may be placed, in the manner above described, in an inflamed conjunctiva. If the preparation is taken out after twenty-hour hours, and studied immediately on the warm stage, we are able to satisfy ourselves that, in those parts which exhibit the characteristic silver staining, young cells are actually found in the canaliculi, and pass along them.

Of mammalia, young rabbits answer best for studies of the cornea. Inflammation is excited by the same methods. The results are also similar. In a cornea excised twenty-four hours after thorough cauterization, and stained with gold, parts are found in the strips which are obtained by the method previously described, in which the canaliculi assume the character of channels of even width, which, as well as the cell cavities, are lined with chains of small cells, arranged in linear series, so as to resemble endothelial elements. From these appearances, we are justified in concluding that both the bodies and the processes of the cornea corpuscles have split into young elements, changing, at the same time, their form.

Inflammation of the Tongue of the Frog.—In the tongue, cell division can be studied both in the corpuscles peculiar to the organ and in migratory cells. For this purpose, the tongue is prepared as for the study of the circulation. The mucous membrane covering the large lymphatic sac of the under surface is snipped off with curved scissors. The observation is necessarily tedious, often lasting for forty-eight hours. It is therefore desirable to replace the tongue in the mouth for a time, after each examination.

Inflammatory Changes in the Tadpole's Tail. — The inflammatory changes which take place in branched cells may be studied in those of the tadpole's tail. In a curarized tadpole, the required degree of irritation can be produced either by simply pencilling the surface, or by allowing a drop of ammonia to fall on it from a capillary pipette, or, finally, by piercing it with a needle. The research must be continued often for many hours. The results are similar to those observed in the cornea, and may be studied either in the fresh state or in gold preparations.

ADDENDUM TO CHAPTER V.—p. 71.

Gerlach's method of demonstrating the relation between the ganglion cells and the network of non-medullated nerve fibres in the spinal cord:—Longitudinal sections, which must be as thin as possible, are made through the anterior horns of the grey substance of a perfectly fresh spinal cord of the calf or ox; these are transferred as they are cut, into very dilute solution of bichromate of potash (one part in 5,000—10,000), and allowed to remain for two or three days in a cool place. Thereupon they are placed for twenty-four hours in very dilute solution of carmine; they are then washed in distilled water, teased out superficially on the object-glass, and mounted in glycerin. I have also found it possible to demonstrate the extremely fine network of non-medullated nerve fibres, with the greatest distinctness, in teased preparations of sections of the grey substance of the spinal cord of the calf, after maceration for two or three weeks in one per cent. solution of bichromate of potash.

ERRATA.

p. 15, line 38, after the words "Figs. 2 and 3," add "and 16."
,, 18, ,, 31, for XIV. read XV.
,, 20, ,, 2, after the word "membranes" insert a comma.
,, 26, ,, 31, for Reta, read Rete.
,, 57, ,, 35, for 6°C. to 8°C., read —6°C. to—8°C.
,, 75, ,, 21, after the word "demonstrated" insert lines 38 to 44 on p. 81, from the words "In preparations" to "ganglion cells."
,, 78, ,, 32, after the words "in tufts" insert lines 13 to 20 on p. 79 from the words "Their general direction" to the end of the paragraph.
,, 79, ,, 12, omit the words "which have the following characters."
,, 82, ,, 30, for "sixty" read "ten."
,, 88, ,, 40, for "6° to 20°C." read —6° to—20°C.
,, 94, ,, 6, for "twelfth" read "twelve."
,, 104, ,, 42, for "left" read "right."
,, 123, ,, 7, for VIII. read V.
,, 126, ,, 6, for "ten" read "one-tenth."

PHYSIOLOGY.

PART I.—BLOOD, CIRCULATION, RESPIRATION, AND ANIMAL HEAT.

By Dr. BURDON-SANDERSON.

CHAPTER XV.

THE BLOOD.

Section I.—The Liquor Sanguinis, or Plasma.

The blood is not a liquid, in the strict sense, but consists of coloured and colourless corpuscles suspended in liquor sanguinis. It is necessary, in order to examine the liquor sanguinis, to separate the corpuscles from it by mechanical methods—*i.e.*, by subsidence and decantation, or filtration. As, however, it is not possible, under ordinary circumstances, to remove blood from the body without its undergoing that remarkable change which we call coagulation, neither of these methods can be applied to the blood unless by some means or other it can be kept in a fluid state during the process of filtration. The earliest successful attempt to accomplish this was made by Johannes Müller. His experiment consists in allowing a frog to bleed into a solution of sugar (half per cent.), and then rapidly filtering the mixture. The large corpuscles of the frog's blood are retained, and the liquid passes transparent, and free from corpuscles. After a time it solidifies to a trembling jelly, which eventually contracts into a clot surrounded by serum. This experiment was, for a long period, the only proof of the existence in the blood of a liquid possessing the properties of plasma—that is, of the fact that the liquor sanguinis solidifies when left to itself, quite independently of the corpuscles. It does not, however, enable us to study the properties of this liquid completely, because in Müller's filtrate it is diluted with saccharine solution.

1. **Filtration of the Blood of the Frog.**—Of three test tubes (Fig. 190), each capable of holding about two drachms of liquid, No. 1 is filled to about one-fifth of its depth with a solution of sulphate of soda obtained by mixing one volume of saturated solution with one of distilled water; No. 2 contains about half a drachm of half per cent. solution of sugar; No. 3, half per cent. solution of chloride of sodium. Several frogs are then selected, in each of which the pericardium is exposed and divided as directed in § 46, and a snip made in the ventricle with fine scissors, the integument having been dried with filtering paper before making the first incision. The blood is allowed to flow into No. 1 until four times as much blood has been added to the quantity of solution as the tube previously contained. To each of the liquids in No. 2 and in No. 3 an equal volume of blood is added. Each of the liquids is gently agitated and then thrown on a filter made of strong close-fibred paper prepared for its reception, and correspondingly numbered. In each instance we obtain a clear and colourless filtrate, the whole of the coloured part of the blood, *i.e.*, the corpuscles, being collected on the filter. The three filtrates have, however, different characters. From filter No. 1 is obtained a liquid which remains fluid at ordinary temperatures, *i.e.*, provided that the room is moderately cool. From filter No. 2 we have a liquid which coagulates immediately. From No. 3 a liquid which coagulates after a time: its coagulation will be much accelerated if it is placed in a bath, at a temperature approaching that of the body.

In the sulphate of soda filtrate the appearance of a clot is postponed indefinitely. It is, however, not the less certain that it really contains the immediate principles of which fibrin, the material of the gelatinous mass seen in the other tubes, is formed. This may be demonstrated by diluting the liquid with distilled water. If the original solution had been saturated, water might have been added gradually for some time without producing any apparent change. In the present instance, the solution employed contains one part of saturated solution to one of distilled water. If water is added to the mixture in the proportion of one-fifth of its volume, it is sufficient to render it coagulable, whereas six or seven volumes would have been required if the solution had been concentrated. As, therefore, saturated solution of sulphate of soda contains fifty per cent. of the crystalline salt, this last must, in order to the prevention of coagulation at ordinary temperature, be present in a proportion of not much less than five per cent.

In these experiments it has been shown (1) that the coloured blood corpuscles of the frog are so large that they do not pass through close filtering papers; (2) That in the filtrate, even when it is diluted with its volume of solution of sugar, a gelatinous clot forms immediately, under ordinary temperatures; (3) That the process of coagulation is held in check by certain neutral salts, and in particular by sulphate of soda. A similar influence is exercised by sulphate of magnesia, nitrate of soda, borax, and some other neutral salts.

2. **Separation of the Corpuscles from the Liquor Sanguinis or Plasma in the Blood of Mammalia, by Subsidence and Decantation.**—It is not possible to filter mammalian blood in the way above described; for the corpuscles are so small that they will run through the finest filtering paper. We must, therefore, have recourse to subsidence. The difficulties of separating the liquor sanguinis from the corpuscles by subsidence depends on the length of time which the corpuscles take to settle, as compared with the rapidity with which the blood coagulates. In consideration of both these circumstances we select the blood of the horse as preferable to any other. In horse-blood the specific gravity of the globules is 1105, that of the liquor sanguinis 1027-1028 (Hoppe-Seyler): the difference is considerable, and somewhat greater than in other animals. But it is of more importance still that horse-blood coagulates more slowly than that of other animals.

If blood is received into one of two similar jars from a bullock, into the other from a horse, it is seen that after an hour or two both have coagulated firmly. In the former, the clot is all of one colour; in the latter, it is divided by a tolerably defined horizontal line into an upper colourless, and a lower deeply coloured part, the upper being a little more than half the depth of the other. In the one case the corpuscles have had time to descend through the upper stratum of liquid before it solidified, whereas in the other their descent is anticipated by the coagulation of the plasma. In the horse this appearance is always observed when the blood taken from a bloodvessel is allowed to stand. In other animals, and particularly in man, it occurs only under abnormal conditions (particularly inflammatory fever). It is spoken of as the "buffy coat."

In the experiment above described, the object we have in view has not been attained. The corpuscles have subsided more or less completely, but the plasma no longer exists as such. It has separated into clot and serum. To succeed, coagulation must not only be delayed but prevented—for which purpose there is but one means available, *i.e.*, cold. At the temperature of freezing, coagulation is indefinitely postponed. The blood must, therefore, as it flows from the animal, be subjected to this temperature, and kept under its protective influence. For this purpose a cylindrical vessel made of tinplate, of the form shown in Fig. 191, is used. This vessel is not only surrounded with ice externally, but contains in its axis a smaller cylinder, closed at its lower end, which is also filled with ice. Between the external surface of the smaller cylinder and the internal surface of the larger, there is an interval which does not exceed half an inch in width, so that the whole of the liquid which occupies it is kept at freezing temperature. In the course of two hours or less the blood has separated into two layers, of which the lower contains all the corpuscles. The upper stratum consists entirely of plasma—a liquid which in its general aspect resembles ordinary serum, but is not so transparent. The most obvions as well as the most important property which it possesses is that of coagulation. So long as it is kept at 0° C. it remains liquid; but if the temperature is allowed to rise even a few degrees above freezing point, the whole mass is converted into a gelatinous clot.

3. **Experiments Illustrative of the Properties of Plasma and Fibrin.**—1. Transfer some of the plasma, with the aid of a cooled pipette, to a small narrow test glass, surrounded with ice and water contained in a small beaker. As the ice gradually wastes, the liquid becomes gelatinous. The surface by which the mass adheres to the glass is so extensive as compared with its volume, that the adhesion is permanent. Consequently, if the tube is examined after having been left to itself for several hours, it is found that the plasma has not (as in other cases of coagulation) separated into clot and serum, but that it appears to be entirely semi-transparent and gelatinous.

2. Another quantity of plasma is allowed to coagulate in a wide vessel. At first the process seems to go on in a similar manner, and for a time the mass adheres to the sides of the vessel. Afterwards, as it contracts, drops of serum collect, first on the surface, then between the clot and the sides of the glass. Soon the clot detaches itself wholly from the vessel, at the same time diminishing in volume. Eventually we have a clear liquid (serum) in which an opaque white cast of the beaker floats. As, in consequence of the adhesion of the coagulum to the sides, contraction is more resisted in the horizontal direction than in the vertical; the upper surface always becomes more or less concave.

3. Preparation of Fibrin.—*a*. The clot from 2 is removed from the liquid, divided into small fragments, and washed with water until it is absolutely colourless. In this condition it differs strikingly from the semi-transparent gelatinous mass which is obtained in 1. It is dense, fibrous, and opaque, and extremely elastic. *b*. A fresh portion of plasma is briskly agitated with

a rod of whalebone or other suitable implement. In this case the fibrin is obtained in fine fibres, which may also be rendered white by washing. In *a* the fibrin has passed through a previous condition in which it was gelatinous. In *b* it is obtained directly in the fibrillated state.

4. Some plasma is diluted with one hundred times its volume of ice-cold water, or three-quarter per cent. salt solution, and allowed to stand. After twenty-four hours, it will be found that there are long delicate filaments of fibrin, which stretch across the mass of liquid in every direction, from one side to the other of the vessel in which it is contained. These filaments, the extremities of which adhere to the glass surface, are in the highest degree elastic. If they are separated from their points of attachment, they shrivel up into little lumps of fibrin. If these again be drawn out into lengths, they resume their original form when let go, as completely as a bit of india-rubber would do.

5. The fibrin prepared in 3 is placed in water containing one per thousand of hydrochloric acid. At first it swells out into a bulky hyaline mass. If it is then placed in the air bath, and kept at a temperature of from 40° to 60° C., it wastes away at a rate which varies according to the temperature. In undergoing solution the fibrin has been transformed into another albuminous compound, syntonin or acid-albumin.* If the liquid is carefully neutralized, the syntonin is precipitated, but the precipitate is re-dissolved in a slight excess of alkali or alkaline carbonate.

6. Another portion of the same fibrin is soaked in solution of peroxide of hydrogen. It is then placed on a sheet of filtering paper, which has been previously soaked in tincture of guaiacum. It soon becomes surrounded with a border of blue, in consequence of the oxidation of the guaiacum. Another method consists in first steeping a fragment of fibrin in alcohol, then in tincture of guaiacum, and finally immersing it in the solution of the peroxide: the fibrin becomes blue. The same thing happens if the fibrin is dipped in a mixture of the tincture and the solution. This reaction signifies simply that fibrin decomposes peroxide of hydrogen: it affords no proof of the presence of ozone.

4. **Experiments relating to the so-called Fibrin Factors—Paraglobulin and Fibrinogen.**—In every act of coagulation, fibrin appears to be produced by the combination of two albuminous substances closely allied as regards their chemical characters, both of which are to be found in plasma as obtained by any of the methods above described. Fifty cubic centimeters

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* The ending *in* is adopted here and elsewhere to denote that the word is used in a stœchiological sense. Albumen is white of egg.

or thereabouts of the plasma, which has been kept at a freezing temperature, are added, in a beaker, to five hundred centimeters of distilled water. A current of carbonic acid gas is allowed to pass through the liquid until it becomes turbid; much froth collects on the surface. On discontinuing the current, it is found that a distinctly granular precipitate has been formed. This is paraglobulin. After decanting off most of the liquid, the precipitate is collected on a filter and washed with water saturated with carbonic acid. It is insoluble in water which has been boiled, but soluble in water containing air or oxygen; it decomposes peroxide of hydrogen in the same way as fibrin. It is characteristic of the solution that when mixed with a solution of a substance to be spoken of immediately under the name of fibrinogen, fibrin is produced. This property is denoted by the term *fibrinoplastic,* which is applied both to the substance and to the solution. 4

2. After the precipitate has had time to subside, the clear liquid is decanted off, diluted with twice its own bulk of *ice-cold* water. A stream of carbonic acid gas is again passed through it. At first it remains clear, but after a time a somewhat viscid scum begins to collect on the surface of the liquid and on the sides and bottom of the glass. This precipitate is fibrinogen. This process involves an immense expenditure of ice, and occupies a great deal of time.

3. Fifty cubic centimeters of serum of ox-blood are mixed with half a litre of distilled water. A stream of carbonic acid gas is passed through it as before. A granular precipitate is formed, which, like that obtained from plasma, is fibrinoplastic.

4. Fifty cubic centimeters of hydrocele fluid or pericardial fluid are diluted with water and treated with carbonic acid gas as before. A slimy white substance is formed in very small quantity, which collects on the surface of the liquid and on that of the glass.

5. The granular precipitates in 1 and 3 may be obtained in the same form by adding to the same diluted liquids acetic acid, the quantity of which must be so small that the liquid still retains a trace of alkalinity. The precipitate has the characters described in 1.

6. Twenty cubic centimeters of filtered hydrocele or pericardial fluid are placed in a beaker in the air bath at a temperature of 40° C. The liquid does not coagulate, but on adding serum a firm clot is formed.

7. A second quantity of the same liquid which has been ascertained by the preceding experiment to be fibrinogenic, *i.e.*, to have the property of coagulating on the addition of a

fibrinoplastic liquid, is saturated with pure chloride of sodium by adding the salt gradually in fine powder. As the point of saturation approaches, the previously clear liquid becomes cloudy, and on standing, a flocculent deposit separates. This deposit is fibrinogen. It must be collected on a filter and well washed with saturated solution of common salt. If the substance so prepared is dissolved in a small quantity of distilled water, and the liquid filtered, a clear solution of fibrinogen and chloride of sodium is obtained. It possesses the property of coagulating on the addition of serum, especially at a temperature approaching that of the body.

8. Filtered serum of blood treated in precisely the same way yields a similar product containing paraglobulin. The filtrate obtained determines coagulation in hydrocele liquid when added to it. Coagulation may be also expected to occur when the fibrinoplastic filtrate obtained in 8 is added to the fibrinogenic filtrate obtained in 7. The result of this experiment is, however, uncertain.

9. If plasma is saturated with chloride of sodium in the manner above described, a precipitate is obtained which contains both paraglobulin and fibrinogen. If this is washed with saturated solution of salt as before, dissolved in distilled water, and rapidly filtered, a clear fluid passes through, which after a while coagulates, and which has the characters of fibrin.

10. If the transudation liquids above mentioned cannot be obtained, a liquid may be prepared by adding to plasma a solution of a neutral salt, such as sulphate of magnesia or sulphate of soda, so as to prevent coagulation. If the quantity of neutral salt added is just sufficient for the purpose, the addition of a little paraglobulin at once determines the formation of a clot.—Blood is received directly from the circulation into one-third of its volume of ice-cold saturated solution of sulphate of soda or of sulphate of magnesia. The mixture is allowed to stand in ice till next day, in order that the corpuscles may completely or in great measure settle. The clear liquid (plasma and neutral salt solution) is then removed by decantation with a capillary syphon, and used as follows:—*a* A small quantity is placed in an eprouvette, in the warm chamber, at 40° C. *b* Other quantities are diluted with proportions of distilled water, varying from 4 parts to 10 parts, and kept at the ordinary temperature. *a* Coagulates at once. Of *b* the more dilute coagulate spontaneously, even at the ordinary temperature. To those that do not so coagulate, paraglobulin is added, when it is found that in the more concentrated quantities the addition determines the formation of a clot. Kühne recom-

mends for this experiment a solution of sulphate of magnesia containing 1 part of the salt to 3½ of water. Plasma mixed with this solution in the proportion of 3 parts to 1, and then diluted with 8 parts of water, coagulates on the addition of paraglobulin.*

11. Diluted plasma which has been treated with carbonic acid gas does not coagulate, even when shaken with air and subjected to the temperature of the body (40° C.).

From the above experiments we learn that plasma contains two albuminous compounds, precipitable by carbonic acid gas and by acetic acid; that one of them (paraglobulin) exists alone in serum in considerable quantity; that the other (fibrinogen) exists alone in liquids effused into *uninflamed* serous cavities in very small quantity; that when paraglobulin is added to these effusion-liquids they become coagulable, just as serum may be made coagulable by the addition of fibrinogen.

5. **Heynsius' Experiment.**†—From the properties of blood plasma demonstrated in the above experiments, we are apt to infer that this liquid is the exclusive source of the fibrin formed when blood coagulates. There is reason, however, for believing that a very considerable quantity of fibrin-producing material is contained while the blood is circulating, in the coloured or colourless corpuscles, for it can be shown that if these elements are separated as completely as possible by subsidence and decantation from a known quantity of blood, and added to a similar quantity of serum, this serum acquires the property of coagulating; and the quantity of fibrin produced bears a very considerable proportion to the whole quantity which the blood would have yielded. Fifty cubic centimeters of blood are received directly from the vein of a horse or ass into a measuring tube surrounded with ice. The blood is immediately afterwards poured into a tall narrow glass cylinder, which already contains half a litre of a two per cent. solution of common salt, previously cooled by standing in ice. In this vessel the mixture is allowed to remain until the corpuscles have subsided, after which the liquid must be drawn off with the aid of a capillary pipette or syphon. The remainder is then mixed with a similar quantity of salt solution, again left to itself surrounded by ice, and the process repeated. Fifty centimeters of serum of ox-blood previously prepared, having been then added to the corpuscles which remain at the bottom of the vessel, the mixture is placed in water at a temperature of 40° C. After two or three minutes coagulation takes place. The clot is collected and

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* Lehrbuch der physiol. Chemie, p. 172.

† Pflüger's Archiv. B. III. p. 419.

washed, dried, and weighed. In the meantime the fibrin yielded by an equal quantity of blood is determined. On comparing the weights, it is found, as before stated, that the coagulum obtained from the mixture of serum and corpuscles alone, is nearly equal to that obtained from the whole blood (corpuscles and plasma). It has been further shown by Heynsius, that if blood is received in an ice-cold, half per cent. or one per cent. solution of common salt, the quantity of fibrin yielded by the plasma is much less (so to speak) than it ought to be, *i.e.*, much less than that yielded by a corresponding quantity of blood. This fact, taken in connection with the result of our experiment, leads us to regard it as probable that in circulating blood, the liquor sanguinis contains less of the fibrin factors than it does immediately after its removal from the body. If this inference is correct, there can be little doubt that it somehow or other, in leaving the living vessel, acquires fresh properties of coagulation from its formed elements. Heynsius believes that the coloured blood disks are alone concerned in this action, and attributes it to the discharge into the plasma of certain of their constituents. His results are, however, quite as consistent with the belief that the colourless elements are the chief agents, in favour of which several facts may be demonstrated. Vaccine and blister fluid are both coagulable; they contain no coloured blood corpuscles, but always many colourless corpuscles. If the process of coagulation is watched in either of these liquids under the microscope, it is seen, not merely that it begins from these elements, but that it occurs nowhere in the liquid excepting where they are present. Again, if a ligature is drawn through a vein in which blood is circulating, as, *e.g.*, through the external jugular of a rabbit or guineapig, and allowed to remain there for a time, and then removed and examined microscopically, it is found that the threads of the ligature are crowded, and its surface encrusted, with colourless corpuscles. These bodies are held together by fibrin, which appears to grow from their surface into the blood-stream.

SECTION II.—CONDITIONS WHICH AFFECT THE COAGULATION OF THE BLOOD.

Although the circulating blood contains either in its coloured corpuscles or plasma both the fibrin factors, *i.e.*, the immediate principles necessary for its coagulation, it does not coagulate. In other words, the blood, so long as it forms part of the normal living body, contains no fibrin. This remarkable fact is dependent on the maintenance in the corpuscles of those chemical changes which constitute their life. And inasmuch as these changes cannot continue in the absence of the physical and chemical conditions to which the blood is subjected, so long as it is contained in healthy blood vessels, any derangement of those conditions leads to the formation of a clot. It can be proved experimentally (1) That blood does not coagulate in the living heart or in a living blood-vessel, even when the circulation is arrested; (2) That although normal blood ordinarily coagulates as soon as it is withdrawn from the body, there are certain circumstances under which the act of coagulation either does not take place, or is accomplished in so imperfect a manner, that the clot is scarcely recognizable as such.

6. The following is a modification of an experiment of Brücke, devised by my friend Dr. Durante. In a rabbit, two small incisions are made across the course of the external jugular vein, (*see* § 48) one near the clavicle, the other near the origin of the vessel—great care being taken not to go deeper than is necessary in order to see the vessel through the fascia. A small needle is then passed under the vein near the proximal incision, in a direction at right angles to that of its axis, and corresponding to that of the incision, but deeper. A second needle is then laid in the course of the incision, and drawn tightly towards the first by a ligature at either end, by which means the blood-current is entirely arrested, while the coats of the vein are absolutely protected from injury. A second pair of needles is then inserted at the distal incision, and secured in a similar manner, so as to shut in the blood with which the vein becomes distended after the tightening of the first ligature. After the lapse of a couple of days, the ligatured portion of the vein is exposed at some part of its course, and punctured with a glass pipette, by means of which the blood is withdrawn from it by suction in a perfectly liquid state. On removing the needles the natural circulation is at once restored. This result, however, is only obtained when the greatest care is used to avoid injury to the coats of the vein. This may be readily proved by repeating the experiment (which, in a practical point of view, is of great importance) in a different way. If, instead of using needles, ordinary ligatures are placed on the points indicated, a coagulum is formed, so that on pinching the vein no blood flows. On opening such a vessel it is found to be occupied by two clots (thrombi), each of which is thickest and firmest at the ligature, and becomes thinner and looser towards the middle of the deligated part. Dr. Durante has shown that, in this experiment, the absence of coagulation depends on the integrity of the endothelium. Wherever the endothelium of a vein is irritated so as to undergo germination, a clot is formed which is co-extensive with the alteration of the endothelial elements.

7. The arterial trunks leading from the heart of a frog or

tortoise are first tied, and then (as soon as the heart has become distended) the venous trunks. The heart full of blood is removed from the body and suspended in a small flask by one of the ligatures. The flask is allowed to stand so long as the heart continues to pulsate. If, then, before the pulsations have entirely ceased, the blood is allowed to flow from the heart by removing the arterial ligatures, it is seen to be fluid. As soon as it escapes it coagulates. This is also an experiment of Brücke.

8. **Recklinghausen's Experiment.**— A small porcelain crucible is heated to redness, and allowed to cool without removing the cover. The pericardium of a frog is then exposed and divided, and a snip made in the ventricle with absolutely clean scissors, the frog being held in such a position that the blood discharged from the wound in the heart may be received in the prepared crucible *without coming in contact with the external surface of the body.* The quantity of blood used should not exceed ten drops. The crucible (without its cover) is then placed on a ground-glass plate, and covered with a wide bell-glass, the edge of which is also ground, so that it fits the glass plate perfectly. The blood coagulates immediately, but during the course of the next twenty-four hours it *appears* to become liquid again. If the experiment has been carefully performed, the blood remains unaltered (its colourless corpuscles retaining their vital activity) for many days: it is, however, necessary to renew the air contained in the bell-glass, by lifting it carefully from time to time. This experiment may be also made with mammalian blood, provided that a temperature is maintained equal to that of the body, for which purpose v. Recklinghausen uses an air bath furnished with a Bunsen's regulator. The capsule is heated to redness, because, if it were not so, the organic matter adherent to the surface of the porcelain would determine changes in the blood, which would be fatal to the vitality of its elements. With a similar view every possible precaution is used against other modes of contamination, whether from the air or from surfaces with which the blood is brought into contact. The liquefaction of the coagulum in the preceding experiment is only apparent. To prove this, the process must be observed microscopically under otherwise similar conditions. The following method, suggested by certain experiments of Schlarewski (who, however, does not appear to have understood their significance), I owe to my assistant Mr. Schäfer. Several very thin walled capillary tubes, not more than $\frac{1}{4}$ millimeter in diameter, are filled with blood as it flows from the artery of a frog, and at once placed under the No. 9 immersion objective of Hartnack. The contents of the tube can be seen with perfect distinctness. At first the whole of the space enclosed in the tube is occupied by coloured

blood disks. After a few minutes it is seen that coagulation has occurred, and that the cylindrical mass in which the corpuscles are contained is separated from the glass, by a transparent border in which there are no corpuscles. Next, the colourless corpuscles begin to squeeze themselves out of the coagulum and swim in the serum (*see* Fig. 192). From the activity of the amœboid movements which these corpuscles exhibit immediately after their expulsion, the observer is inclined to attribute their escape from the clot to these movements; this notion is, however, proved to be erroneous by what follows. In a short time (usually about forty-five minutes after the commencement of the observation), the coloured corpuscles begin to participate in the process, and escape from the still sharply-defined edge of the clot in such numbers that the liquid becomes so crowded with them, that microscopical examination is no longer possible. If now the tube is removed from the stage and placed vertically, it is seen, after a time, that the corpuscles subside to the bottom of the tube, leaving a clear space containing serum above. Here, then, we have a process which we might at first sight be disposed to regard as a resolution of the coagulum: the appearance is, however, deceptive, for if the tube is discharged into a watch-glass and examined under a low power, the coagulum is easily found as a thin cord of fibrin floating in the liquid. In short, the whole process of emigration of the corpuscles and liquefaction of the clot is the consequence of the contraction of a reticulum of fibrin of such extreme looseness, that it is incapable of retaining the corpuscles in its meshes.

9. The two experiments last related prove, as regards the blood of the frog, that, under certain conditions, coagulation occurs very imperfectly, even though the blood be removed from the body, and consequently that Brücke's inference, that the circulating blood is prevented from coagulating by the influence of the living vessel, need no longer be maintained. The following experiment, devised by Mr. Schäfer, which has been repeated a great number of times in the laboratory of University College, proves this much more conclusively and satisfactorily. A glass tube, three or four inches long, is drawn out at one end into an arterial cannula of the usual form and of suitable size. A frog having been secured in the usual way (*see* § 46) in the prone position, the heart is exposed and the right aorta ligatured. A clip is then placed on the left aorta at its origin from the bulb. The cannula (Fig. 193, *a*) is then inserted and secured in the left aorta, and the tube supported vertically by a suitable holder. This done, and the clip having been removed, the blood is allowed to flow into the tube. It rises to a height which varies according to the vigour of the animal and the quantity of blood which

its vascular system contains, the blood column oscillating with the contractions of the heart. If now the tube is left to itself, no coagulation takes place. In a very few minutes the corpuscles begin to subside, leaving an upper layer of clear liquid, the depth of which gradually increases. If it is removed with a capillary pipette and submitted to examination, it is found to possess all the properties which are characteristic of plasma. It contains scarcely any coloured but a considerable number of colourless corpuscles.

SECTION III.—THE COLOURING MATTER.

10. Methods by which the Blood can be rendered Transparent or Laky.—It has long been known that, when water is added to blood in quantity, the blood corpuscles are apparently dissolved in the diluted liquor sanguinis. This solution is, however, only partial; for, if the liquid is examined under the microscope, each corpuscle is seen to be represented by a colourless spheroidal residue. This residue was formerly described as the membrane of the corpuscle, rather in conformity to the notion that, being a cell, it must have a membrane, than because the structure in question possessed membranous characters. We now recognize it, not as a membrane, but as the porous structure fully described in the histological part as the œcoid.

There are many other methods by which the zooid may be compelled to relinquish its dwelling without altering the density of the serum at all. So long ago as 1851, Dr. De Chaumont discovered that the vapour of chloroform had this effect. That of ether acts in the same way, but not so rapidly. More recently, it has been shown by Rollett that the same effects are produced by freezing, as well as by electrical discharges and induction currents. In all these cases (as has been already seen as regards some of them) the blood undergoes a remarkable change of appearance. In the natural state, blood, even in the thinnest layers, is opaque. One may judge of this by looking at it either by transparent light (as, *e.g.*, in a very thin capillary tube) or by reflected light, spread out in a thin layer over the surface of a porcelain capsule. In the former case the blood presents the appearance of a solid-looking band in the axis of a glass rod, in the latter it appears as a bright scarlet patch, completely concealing the white surface, and obscuring the light which would otherwise be reflected by it. If, however, the blood has been subjected to any of the processes above mentioned, the appearances it presents in the two cases are materially altered. The blood in the tube looks bright, because it is translucent, whereas that on the porcelain looks as dark as if it were venous, because the corpuscles from which the light shone, reflected by countless convex surfaces, are now scarcely more refractive than the liquid in which they are immersed. In other words, blood in the natural state has the character of an opaque pigment, such as vermilion; whereas in the altered state it resembles a lake, —a fact which Rollett, who, as I have stated, has studied these changes with great exactitude, expresses by the terms *deckfarbig* and *lackfarbig*, as applicable to the former and the latter respectively. Blood may be rendered transparent or laky by exposing it either to extreme cold or to a temperature a little above 60° C.; by subjecting it to the action either of induced currents or of shocks of frictional electricity. A similar effect, as already stated, is produced by the addition of water and of various other liquid reagents, such as ether, chloroform, and solutions of the bile acids in combination with alkaline bases.

11. **Action of Cold.**—A platinum capsule containing a couple of cubic centimeters of defibrinated blood is exposed to a temperature of —6° to —10° C.,* by placing it in a vessel previously filled with alternate layers of pounded ice and salt, and leaving it in contact with the freezing mixture until it is completely frozen through. The solid mass of blood is then slowly thawed and poured into a beaker, which should be of such size that the blood contained in it is not more than half an inch deep. If readily crystallizable blood has been employed, as, for example, that of the guineapig, a sediment of crystals forms on the bottom. It is seen from the first that the freezing has completely altered its appearance. It has become darker in colour, and if we place some of it on the surface of a white plate with a pattern on it, the pattern is visible with more or less distinctness through it, whereas if ordinary blood were employed it would be completely concealed. It is scarcely necessary to add that the crystallization is dependent on the discharge of the hæmoglobin from the corpuscles into the liquor sanguinis.

12. **Action of Heat.**—(Method of Max Schultze.) This is a method which is only applicable to small quantities of blood. In experiments with the warm stage (*see* Chap. I., p. 6) Max Schultze found that when blood is heated from 60° C. to 64° C., the blood corpuscles dissolve in the plasma. The same effect is produced if a small quantity of blood is subjected to similar temperatures in a hot chamber, furnished with Bunsen's

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* The effect of subjecting blood to the temperature of a freezing mixture was first studied by Hewson. His experiment was similar to that described in the text. His purpose was to show that cold is not the cause of coagulation. He was not aware that frozen blood loses its opacity.

regulator. Here, as in the former case, if the blood is derived from an animal in which the hæmoglobin crystallizes readily, crystals are obtained. According to Preyer, remarkably fine crystals of hæmoglobin may be prepared by warming the coloured corpuscles separated by subsidence and decantation from the defibrinated blood of the horse, in the manner above described. To ensure success, care must be taken to maintain the temperature of the quantity of blood operated on within the limits of temperature above mentioned.

13. **Action of Electricity.**—The effects both of shocks of frictional electricity and of induced currents have been described in the histological part. To what is there stated, it may be added, as regards induced currents, that the most marked effects are produced when the current is most analogous in its characters to a discharge of statical electricity, and, consequently, that the direct induced current which accompanies the opening of the primary current is more effectual than the inverse one. In the results observed, it is important to distinguish between the direct action of the shock or shocks on the corpuscles, and the electrolytic action indicated by the liberation of gases at the tinfoil points (*see* Fig. 194). In so far as electrolysis occurs, the results may be in part attributed to the development of acid reaction at the positive pole, consequent on the decomposition of the salts of the blood. A distinction ought also to be drawn between those effects which are only produced when the corpuscles are in a living state, and those which are manifested also in dead blood. The discharge of the colouring matter from the corpuscles is a phenomenon of the latter class, but there are other effects which manifest themselves only when the blood employed still retains its vital properties.

14. **Action of Water on the Blood.**—The mode of action of water on the corpuscles is fully described in Chapter I. The colouring matter is entirely discharged, and probably the greater part of the globulin. That the whole is not expelled seems evident from an old experiment, made more than twenty-five years ago by Dr. Buchanan, of Glasgow, who observed that the solid residue left behind, even when repeatedly washed with distilled water, still retained the power of determining coagulation in serous effusion-liquids, when added to them in small quantity. Again, when blood which has been acted on by water is subjected to a stream of carbonic acid gas, the stromata of the corpuscles show changes which indicate that they still retain a substance precipitable by that gas.

15. **Action of Crystallized Ox-bile.**—On the addition of a dilute solution of "bile crystals," *i.e.*, crystals of glyco-cholate and tauro-cholate of soda to blood, a great number of the corpus-

cles are dissolved, so that the blood becomes distinctly laky; and if it is derived from a suitable source, and not too much diluted, the colouring matter crystallizes. On this fact one of the numerous methods of obtaining hæmoglobin is founded. With reference to the mode of obtaining "bile crystals," *see* Chap. XXXVI.

16. Preparation of Hæmoglobin.—Any method by which the colouring matter can be caused to quit the corpuscles without undergoing chemical change, or, in other words, any of the methods by which the blood can be rendered transparent or laky, may be used for obtaining crystalline hæmoglobin. Many of these methods yield the product very readily, when the blood is derived from one of those animals in which the colouring matter is prone to crystallize. There are, however, only one or two of them by which pure hæmoglobin can be obtained in considerable quantity.

Thus by the method of freezing, large well-formed crystals can be obtained from the blood of the guineapig or dog. In like manner the blood of the same animals crystallizes readily after it has been rendered laky by warming or by the transmission of induction shocks.

When it is intended to prepare considerable quantities in a state of purity, it is best to employ water as a solvent, and then to determine crystallization in the liquid by the addition of alcohol, in such proportions that the mixture is only just capable of retaining the colouring matter in solution. To ensure success, it is to be borne in mind that the colouring matter crystallizes as oxyhæmoglobin (*sec* § 17), that crystallization is much impeded by the presence of non-crystallizable organic compounds, particularly albumin, and that hæmoglobin is prone to undergo change when exposed in solution to temperatures above that of freezing. To ensure complete oxidation, the blood must be freely exposed to air. To obviate the interfering influence of albumin, the colouring matter must be derived, not from the whole of the blood, but in as far as possible from the corpuscles alone. To obviate the risk of chemical change, *i.e.*, of the splitting of the hæmoglobin into other products, the liquids must be subjected, so far as possible, during the whole operation to a low temperature. These indications are fulfilled in the following process, devised by Preyer, which gives good results, when the weather is cold and when blood is used of which the colouring matter is comparatively insoluble in water at 0° C., *e.g.*, that of the dog or cat. The hæmoglobin of the blood of the horse, on the other hand, is very soluble at all temperatures. It cannot therefore be prepared by Preyer's method. Blood to be employed is allowed to flow from a vein or artery into a porcelain capsule. It is then placed in a cool cellar to coagulate. On the following day most of the serum is poured off, and the remainder removed with the aid of a pipette. The clot is then cut into small fragments and placed on a filter of fine calico, on which it is washed repeatedly with *ice-cold* distilled water, until the washings give scarcely any precipitate with a solution of corrosive sublimate. [This indicates that the clot is tolerably free from serum-albumin. The water must be ice-cold, because at freezing temperature hæmoglobin is sparingly soluble.] Then on the filter the clot is treated with distilled water at a temperature of about 35° C., the filtrate being allowed to drop into a measure-glass cooled in ice. It is of great importance that this part of the process should be carried out with as little loss of time as possible. I have found it a good plan to enclose the clot in the filter, and then to knead it repeatedly in *small* quantities of warm water contained in a capsule; the products of all the extractions being collected on the same filter, and received in the cooled beaker. A measured portion (say ten cubic centimeters) is then transferred, with the aid of a pipette, to a test-glass, to which alcohol is added drop by drop from a burette. The precipitate formed by the first drops of alcohol redissolves on shaking or stirring: as more alcohol is added the precipitate at last remains undissolved. [By this means the proportion of alcohol required, in order to diminish the solvent power of the liquid sufficiently to render it prone to crystallize, is determined.] Alcohol is then added to the whole liquid, in proportion somewhat less than is required to produce a permanent precipitate. The clear solution on being left to itself, surrounded with iced water, soon begins to crystallize. The crystals are separated by filtration and washed on the filter with ice-cold water containing a little spirit, and subsequently with ice-cold water alone. To obtain the substance in a state of purity it must be subjected to recrystallization. For this purpose the crystals must be dissolved in distilled water at 40° C. and evaporated *in vacuo*, the process being repeated until a product is obtained which on incineration leaves pure oxide of iron without trace of phosphoric acid.

Dr. Gamgee recommends the following process, which was recently communicated to him by Professor Kühne, and has been successfully employed by him on three separate occasions. —Five hundred cubic centimeters of defibrinated blood of a dog are mixed in a flask with 31 c. c. of pure ether, and thoroughly shaken at intervals of a few minutes during an hour and a half or two hours. The mixture is then placed in a cellar for about twenty-four or thirty-six hours. The flask containing the lake-red liquid is now surrounded with ice (not a freezing mixture) for twelve hours, at the end of which time it is found to have become converted into a magma of hæmoglobin crystals. Dr.

Gamgee states that the only objection to this method consists in the great difficulty of filtering the crystalline from the viscid serous portion of the mixture. In laboratories where the centrifugal apparatus is to be found, the magma may be placed in tubes and submitted to excessively rapid rotation for three or four hours, at the end of which time the hæmoglobin will have separated as a soft cake from the serum, which can be decanted. Where no centrifugal apparatus can be obtained, the magma of crystals may be diluted by the addition of an equal volume of a mixture consisting of one part of ninety per cent. alcohol and four parts of distilled water. The whole must be filtered through calico, and the soft hæmoglobin freed from the greater part of the adhering water and spirit by being placed on a porous brick and exposed to a current of cold air. Whichever method of separating the crystals is used, they must be purified by recrystallization.

The best method of obtaining hæmoglobin crystals in small quantities, for microscopical purposes, is one founded on the same principles. A teaspoonful of defibrinated blood is treated with a sufficient quantity of water to render it transparent. A quarter of its bulk of alcohol having been added to it, the mixture is introduced into a platinum capsule, and plunged in a mixture of pounded ice and salt. A relatively abundant crop of crystals is obtained. The mere freezing and thawing the blood, as directed in § 11, will also give satisfactory results. Another method consists in passing the vapour of chloroform through the blood, which has always the effect of rendering it laky, and in some animals determines crystallization.

17. Chemical Properties of Hæmoglobin.—*Solubility.*—The solubility of hæmoglobin in water differs according to the species of animal from which it is derived. Thus the colouring matter of the dog and cat are very soluble at 40° C.; sparingly soluble in ice-cold water. That of the guineapig dissolves with relative difficulty at all temperatures, and crystallizes more readily than that of any of the common domestic animals. All kinds of hæmoglobin are more soluble in warm water than in cold. *Diffusibility.*—Hæmoglobin, although crystallizable, is indiffusible. This can be easily shown by placing a solution of blood or hæmoglobin in a diffusion-cell the septum of which is of good parchment paper.* If an animal membrane is substituted, a certain amount of colouring matter passes from the solution into the water. The fact of the diffusibility of hæmoglobin perhaps stands in relation with the enormous weight of its molecule. *Coagulability.*—Aqueous solutions of hæmoglobin coagulate when heated, just in the same way as albumin, and at about the same temperature (64° C.). When this occurs, the hæmoglobin splits into an albuminous compound and an insoluble colouring matter. *Precipitation by Alcohol.*—Small quantities of alcohol may be added to solutions of blood or hæmoglobin without producing any appreciable change. In continuing the addition a precipitate is formed, which at first is redissolved on shaking, afterwards becomes permanent. *Relation to Oxygen.*—In a solution freely exposed to air, the hæmoglobin is always combined with oxygen (oxyhæmoglobin). Consequently, whenever hæmoglobin is spoken of, it is understood to mean oxyhæmoglobin. This oxygen is so loosely combined, that it begins to separate itself from the hæmoglobin as soon as the pressure of that gas in the gaseous atmosphere to which it is exposed falls below a certain point, recently determined by Worm Müller to be about twenty-five millimeters of mercury. So that when blood is subjected to the air-pump, the hæmoglobin it contains begins to part with its oxygen as soon as the pressure is reduced to about a sixth of an atmosphere. This is expressed by saying that the tension of oxygen in the blood is about twenty-five millimeters Hg. Hæmoglobin in solution can be deprived of its oxygen by the addition to the liquid of certain reducing agents (*see* § 18). In animals completely deprived of air, the hæmoglobin of the blood loses its oxygen completely in less than a minute (*see* § 111). This is, no doubt, owing to the rapid accumulation in the blood of oxidizable products. When blood or solution of hæmoglobin is subjected to the barometer vacuum (*see* Gases of the Blood), it parts with the whole of its oxygen. Hæmoglobin has the property of oxidizing tincture of guaiacum. If a drop of concentrated solution of guaiac resin in absolute alcohol is dropped on to filtering paper, and the alcohol allowed to evaporate, and then a drop of solution placed on the brown spot, a deep blue ring is formed round the edge of the drop. This reaction must not be confused with that observed when fibrin steeped in peroxide of hydrogen produces a similar effect. In the latter case, all that is shown is, that fibrin decomposes the peroxide; in the former, the reaction affords evidence of the presence of nascent oxygen. *Action of Carbonic Oxide.*—Blood which has been saturated with carbonic oxide is entirely deprived of its oxygen, which is replaced by an equal volume of carbonic oxide. On this fact is founded the excellent method of Bernard for the gasometrical determination of the oxygen of the blood (*see* § 32.) The carbonic oxide combines with hæmoglobin in the same way that oxygen does. *Action of Oxide of Nitrogen.*—When oxide of nitrogen is passed through a solution of blood which has been freed from oxygen, by subjecting it to an atmosphere of hydrogen in such a manner as to exclude atmospheric air during the process, the dark blood acquires a carmine colour. Here, as in the case of carbonic oxide, a new compound is formed with hæmoglobin, which crystallizes in the same form as oxyhæmoglobin. The solution, however, undergoes no change when treated with reducing agents. *Action of Nitrites.*—Dr. Gamgee has shown that the blood of animals poisoned with nitrites, as *e.g.*, nitrite of amyl, assumes a chocolate colour. This colour may be observed strikingly if a few drops of nitrite of amyl are added to a solution of hæmoglobin. The colour of the latter almost instantly becomes brown. On adding reducing agents to solutions so altered, reduced hæmoglobin (*see* § 18) appears—a fact which seems to square best with the assumption that the action of the nitrites on hæmoglobin is to peroxidize it, and that on reduction, oxyhæmoglobin is first formed, then reduced. The precise nature of the reaction is still matter for investigation.

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* For method of preparing and testing a diffusion-cell, *see* Chapter on Chemical Methods.

18. **Optical Properties of Hæmoglobin.**—*Crystals.*—The crystals are doubly refractive, *i.e.*, they look luminous when examined with the aid of the polarization microscope (*see* Part I., Chap. IV.), between crossed Nicols. They shine in sunlight with a lustre compared by Preyer to that of silk. When formed in liquids freely exposed to air or oxygen, they are of the colour of arterial blood, but have the wonderful property of becoming dark without altering their form when placed *in vacuo* at a low temperature. They then exhibit two colours, looking green along the *arêtes,* purplish-red elsewhere. On the admission of air or oxygen, the colour is restored. If a glass plate to which crystals of hæmoglobin adhere is placed in front of the slit of the spectroscope, two characteristic absorption bands (Hoppe-Seyler) are seen in the yellow between the Frauenhofer's lines D and E (*see* Fig. 195, 1). *Solution.*—The bands just mentioned are also seen when solution of hæmoglobin or of blood corpuscles is placed in the same position: they can be distinguished even when the solution contains only one ten-thousandth of its weight of colouring matter. The bands differ, however, in their characters according to the degree of dilution. According to the experiments of Preyer, solutions varying in strength from one to five per 10,000, show both bands faintly; in solutions of six per 10,000, it can be distinguished that the band next the line D is the darker of the two, the other being broader and fainter (*see* Fig. 195, 5); in solutions of thirty per 10,000, the violet end of the spectrum is completely absorbed, and the blue partially. As the concentration is increased the two bands approach each other, until finally (when the solution contains seventy per 10,000) they form a single band, while the whole of

the more refrangible rays are absorbed, so that the spectrum does not extend beyond the limits of the green (*see* Fig. 195, 6).

In 1862 it was discovered by Stokes that hæmoglobin exists in the blood in two states of oxidation, which are distinguished alike by colour and by the spectroscope; that the oxygenized hæmoglobin, or (as it has since been called) oxyhæmoglobin, is deprived by reducing agents of its oxygen, and that when it has been so reduced, it can be restored to its original state by agitation with air. The nature of the change of colour is expressed in two facts, which can be observed with the aid of the spectroscope. The first is, that when solutions of hæmoglobin, or of blood, are deprived of oxygen, either by placing them *in vacuo* or by the addition of reducing agents, the more refrangible rays (blue and violet) are much less absorbed, and the green more absorbed than they were before. The second fact is, that in solutions so concentrated that most of the spectrum is extinguished, the last colour which is transmitted is orange-red if the blood is arterial, red if it is venous. These two facts may be shortly expressed by saying that the colour of arterialized blood consists of orange-red *plus* green, of venous blood-red *plus* blue.

These differences, however, are not the most remarkable which are observed when oxydized and reduced solutions of blood or its colouring matter, are compared spectroscopically. The most striking change produced by reduction relates to the two bands of absorption in the yellow part of the spectrum which have been already mentioned. This change is most readily demonstrated by following the directions given by Stokes in his original paper. A solution of protosulphate of iron, to which a sufficient quantity of tartaric acid has been added to prevent its being precipitated by alkalies, is rendered decidedly alkaline by the addition of ammonia, and is introduced into the solution of blood. "The colour is almost instantly changed to a much more purple red, as seen in small thicknesses, and a much darker red than before, as seen in greater thickness. The change of colour, which recalls the difference between arterial and venous blood, is striking enough, but the change in the absorption spectrum is far more decisive. The two highly characteristic dark bands seen before, are now replaced by a *single* band, somewhat broader and less sharply defined at its edges than either of the former, and occupying nearly the position of the bright band separating the dark bands of the original solution (*see* Fig. 195, 2). The fluid is more transparent for the blue, and less so for the green than it was before. If the thickness be increased till the whole of the spectrum more refrangible than the red be on the point of disappearing, the last part to remain is *green*, a little beyond the fixed line *b*, in the case of the original solution; and *blue*, some way beyond F, in the case of the modified fluid. If the purple solution be exposed to the air in a shallow vessel, it quickly returns to its original condition, showing the same two characteristic bands as before; and this change takes place immediately, provided a small quantity only of the reducing agent were employed, when the solution is shaken up with air. If an additional quantity of the reagent be now added, the same effect is produced as at first, and the solution may thus be made to go through its changes any number of times." [Stokes, On the Reduction and Oxidation of the Colouring Matter of the Blood. Proceedings of the Roy. Soc., vol. xiii. p. 355]. The same facts can be demonstrated quite as advantageously, and perhaps with greater ease, if the solution of the sulphhydrate of ammonium is substituted for the solution of sulphate of iron used by Stokes. The change is, however, not so rapid : it is accelerated by subjecting the liquid to a temperature of 40° C.

19. **Methæmoglobin.**—If a pure solution of hæmoglobin is left to itself at the ordinary temperature, it gradually loses its brightness, and if it is then examined spectroscopically, it is seen that a new band has appeared in the orange at a point where in ordinary blood there is least absorption. This band is due to the presence of a new colouring matter, called by Hoppe-Seyler methæmoglobin. The same change occurs under other circumstances, *e.g.*, when carbonic acid gas is passed through dilute solutions of hæmoglobin, or when glacial acetic acid is added to dilute solution of defibrinated ox-blood, in extremely small quantity. [In larger proportions, acetic acid determines the formation of hæmatoin.—*See* § 22.] Hæmoglobin undergoes the same transformation when acted on by permanganate of potash. If a crystal of pure permanganate is dissolved in distilled water, and the solution added to very dilute solution of blood, before the slit of the spectroscope, at a temperature of about 25° C., the hæmoglobin bands gradually disappear. In their place we have a spectrum, in which there are not only the band mentioned above, but two others, of which one nearly corresponds in position to the second hæmoglobin band, while the other lies half way between the lines E and F. Methæmoglobin is a substance of which the chemical constitution and relations are imperfectly ascertained. Its presence is indicated spectroscopically in all collections of blood which have been for sometime extravasated within the body, *e.g.*, in thrombi, sanguinolent transudation liquids, &c.

20. **Preparation of the Crystalline Colouring Matters which result from the Decomposition of Hæmoglobin,**

and Demonstration of their Absorption Spectra.—Hæmin. —When dried blood is treated with glacial acetic acid and warmed to the temperature of the body, a solution is obtained which yields crystals of a new colouring matter, of remarkable properties, which has been designated hæmin. The crystals vary extremely in shape, sometimes occurring as rhombic plates, sometimes as rods crossing each other at various angles. They are not soluble without decomposition in any liquid excepting hydrochloric acid, and are so little liable to chemical change that they may be kept for years, exposed to a moist atmosphere, without undergoing any change. Hæmin differs from hæmatin (§ 21) in containing an additional equivalent of hydrochloric acid, on which account it is also called hydro-chlorate of hæmatin. Its carbon, nitrogen, and iron are in the same relative proportions as in hæmatin, but necessarily it contains a little less iron per cent. than that body.

The mode of preparing the so-called Teichmann's crystals—in other words, the mode of obtaining hæmin for the purpose of demonstrating its crystalline form microscopically—has been fully described in the histological part (Chap. I., p. 19). Hæmin may be obtained from blood in quantity, as follows, but the process is one which appears to present great difficulty, as it frequently fails. Defibrinated blood is diluted with a volume and a half of distilled water. The transparent liquid is then precipitated with neutral acetate of lead, for the purpose of separating the albumin. The excess of lead (with respect to which it is desirable to be careful not to add more than is necessary) having been got rid of by the addition of a concentrated solution of carbonate of soda, the liquid is filtered, and the filtrate evaporated to dryness either in the air or *in vacuo*. The dry residue is then finely powdered and rubbed up with fifteen times its own weight of glacial acetic acid, to which a trace of chloride of sodium has been added. The brown liquid thus obtained is introduced into a flask and warmed in the water bath until it is entirely dissolved, and the solution is mixed with five times as much distilled water, and allowed to stand for many days, protected from evaporation. The crystals collect on the bottom of the beaker and may be readily purified by repeatedly treating them with distilled water, allowing them to subside and then decanting. As hæmin contains chlorin, it cannot be prepared from hæmatin unless chlorides be present. When it is prepared from blood, the quantity of chloride of sodium present is sufficient, so that the addition of that salt is not essential. The solution of hæmin in hydrochloric acid gives no characteristic spectrum.

21. **Hæmatin.**—Hæmatin can only be obtained in a state of

perfect purity from the crystals of hæmin, the mode of preparation of which has just been given. The process is simple : the hæmin crystals are dissolved, *i.e.,* decomposed, in ammonia. The solution of hæmatin thus obtained is evaporated to dryness, the residue is then extracted with water, which removes the chloride of ammonium, and dried. The product is pure hæmatin. It is insoluble in water, alcohol, and ether, soluble in alkalies and alkaline carbonates, but not soluble in acids without decomposition.

In the impure state, hæmatin may be obtained in various ways. The change occurs more gradually at ordinary temperatures in solutions of blood, or hæmoglobin, which are decidedly alkaline, whether the alkalinity is derived from potash, soda, ammonia, or their carbonates. Solutions of hæmoglobin which have undergone this last change exhibit, when placed before the slit of the spectroscope, in place of the hæmoglobin bands, a less distinct and paler band on the opposite side of the D line, *i.e.,* in the orange. This change is characteristic of the presence of hæmatin. It is attended with an obvious darkening of the colour of the liquid.

When an alkaline solution of hæmatin is subjected to the action of reducing agents, such as sulphuret of ammonium or protosulphate of iron, it exhibits, when examined spectroscopically, two much more distinct bands (Fig. 195, 4), one of which is exactly opposite the bright space which separates the two hæmoglobin bands; the other, which is less intense, is close to Frauenhofer's line E, *i.e.,* nearer to the blue end of the spectrum than the broader of the two hæmoglobin bands. If the solution is fresh and dilute, and the quantity of the reducing agent small, these bands can be made to vanish by agitation with air, giving way to the so-called oxyhæmatin band above described.—All these facts may be as readily demonstrated in solutions of blood corpuscles, *i.e.,* of cruor, as in solutions of hæmoglobin. Blood rendered distinctly alkaline either by soda, potash, ammonia, or their carbonates, shows the absorption band of oxyhæmatin. After addition of sulphuret of ammonium, this is replaced by the more distinct spectrum of reduced hæmatin.

22. **Hæmatoin.**—When acetic acid is added to blood, the iron of the hæmoglobin is separated and takes the form of a protosalt, and a new colouring matter remains in solution, the spectrum of which was first described by Professor Stokes, and has been subsequently known as acid hæmatin. More recently, Preyer has shown that it is not identical with hæmatin, but with the body to which Hoppe-Seyler gave the name of iron-free hæmatin. It is produced whenever concentrated sulphuric acid acts on hæmatin. According to Hoppe-Seyler, it is prepared by rubbing

up finely powdered hæmatin in concentrated sulphuric acid. A liquid is obtained which is green in thin layers, reddish-brown in thicker layers, and gives a brown precipitate when diluted with water. This precipitate is easily dissolved in ammonia. On evaporating the ammoniacal solution, a bluish-black residue with metallic lustre is left, which is free from iron. It may be obtained in like manner by acting on methæmoglobin by sulphuric acid. The solution of hæmatoin in ammonia exhibits four absorption bands. It is admirably shown by the method recommended by Professor Stokes, *i.e.*, by extracting with ether, blood which has been mixed with acetic acid. The ethereal liquid thus obtained exhibits a four-banded spectrum. Of these bands, three only are easy to recognize—one in the orange, nearer to the red than the reduced hæmatin band; a rather broad band in the green; and a narrow but well-defined one in the blue. (*See* Fig. 195, 3.)

23. **Quantitative Analysis of the Blood, with reference to its Corpuscles, Serum, Fibrin, Hæmoglobin, Albumin, and Salts.**—The following summary of the order of proceeding in the analysis of the blood, will be found sufficient for the guidance of those who have been previously trained in quantitative methods. The student who has not learnt accuracy by practice, in the analysis of bodies of known composition in the chemical laboratory, should not attempt the quantitative determinations relating to the blood or other animal liquids, partly because the operations are complicated, but principally because the operator has no means of detecting his mistakes.—The blood to be analyzed is received in four vessels, the contents of which are as follow:—1. Ten or twelve centimeters of blood are allowed to flow into a weighed porcelain capsule and covered with a weighed watch-glass. After weighing, the blood is evaporated in a water-bath, dried in the air-bath at 120° C., and the residue used for the determination of the total albuminous constituents, fat and salts, as follows:—After standing till it is cool in a receiver over sulphuric acid, it is weighed. The weight, deducted from that of the capsule and watch-glass, gives the *total solids.* The dry residue is then pulverized in a glass or porcelain mortar with common alcohol (Sp. G. 890) and transferred to a small beaker, the mortar being subsequently carefully washed with alcohol, and the washings added to the quantity in the beaker. This done, the contents of the beaker are boiled, and the alcoholic solution thus obtained is poured into a small previously weighed filter. What remains in the beaker is similarly treated with a second quantity of alcohol, which is thereupon poured into the same filter. After carefully washing the filter with boiling alcohol, the filtrate together with the washings is evaporated on

the water-bath, dried at 110° C., allowed to cool over sulphuric acid, and weighed. The weight gives the *solids soluble in alcohol* . . . . *a.*

Distilled water is added to the residue in the beaker, which is warmed in the water-bath. The water-extract is then poured on to the filter last used, and the filtrate collected in a weighed covered capsule, evaporated on the water-bath, dried at 110°, cooled over sulphuric acid, and weighed. The weight, minus that of the capsule, is that of the *solids soluble in water* . . . . *b.*

The remainder on the filter is dried at 110°, and then over sulphuric acid, and weighed repeatedly, till it is found no longer to lose weight. For this purpose it must be enclosed between two watch-glasses, held together by a clamp. The weight, minus that of the watch-glasses, filter, &c., is that of *the insoluble solids*. . . . . *c.*

The fats of the blood are contained in *a*, from which they are extracted by repeatedly treating it with ether and evaporating the ethereal extract. The residue is washed into a small platinum capsule for incineration.

b is incinerated in the capsule in which it was weighed; *c*, with the filter in which it is contained, is incinerated in another capsule. The ash of *a* and *b* represents the soluble salts of the blood, viz., the chloride of sodium (five-sixths of the whole), phosphate, sulphate, and carbonate of soda; chloride and sulphate of potash. The ash of *c* consists of phosphates of lime and magnesia.*

2. A second quantity of twenty-five centimeters is used for the determination of the fibrin. For this purpose a small beaker is used, over the top of which a vulcanized india-rubber cap with a single neck (*see* Fig. 196) can be drawn without difficulty. Through the neck or tubulature, a rod of whalebone, which at its

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* In incinerating, it is of importance that the capsule or crucible should be large enough to hold four or five times as much material as is used. Platinum vessels are preferable. If the substance contains much organic matter, and at the same time much soluble salts, *e.g.*, chlorides, it is necessary to perform the operation in two stages, *i.e.*, first to carbonize the substance, then extract the ash with boiling water, collect the insoluble part on a filter free from ash or containing a known weight of ash. The filter, after careful washing, must be dried at 110° C., and gradually heated to whiteness until the carbon is entirely destroyed. Almost the whole of the soluble salts are contained in the extracts. Thus the decomposition of the alkaline carbonates and chlorides, which occurs at a higher temperature, is avoided. In incineration of the total solids of the blood this interruption of the process is desirable, if for no other reason, on account of the extreme difficulty of getting rid of the carbon in presence of so great a quantity of alkaline salts. If, however, the method described in the text is followed, these difficulties are got rid of in another way. For, on the one hand, the watery and alcoholic extracts contain very little organic matter; on the other, the insoluble residue (*c*) is free from alkaline salts. In both cases, therefore, the incineration can be proceeded with continuously.

lower end widens out into a blade, is grasped by the tubulature. The blood is received into the beaker, covered at once with the cap, and immediately agitated very briskly with the blade of the whalebone, the purpose of the whole arrangement being to prevent loss of weight by evaporation during the process. As soon as coagulation is complete, the beaker and its contents are weighed. The weight, minus that of the beaker its cover and the oar, is that of the quantity of blood used. The cover is then removed and the beaker filled with water, to which a trace of chloride of sodium has been added. After agitation and subsidence the clear liquid is poured off, and the fibrin again treated with as much more water with a trace of salt. The fibrin is then collected on a weighed filter, and washed with distilled water until the filtrate is colourless. The pink fibrin thus obtained is then finally washed on the filter with boiling alcohol, dried first in the air-bath, then over sulphuric acid, and finally weighed.

3. A third portion of blood is received in a similar apparatus, defibrinated, and the defibrinated blood strained through a calico filter and weighed. The filtrate is then mixed in a tall jar, with ten volumes of a solution of salt, prepared by adding nine volumes of water to one of saturated solution. After a day, the corpuscles having subsided, the liquid is decanted off, and replaced by a second similar quantity of saline solution. Again the corpuscles are allowed to subside, and the liquor removed by decantation. The deposit is then washed with water into a porcelain capsule, evaporated on the water-bath, dried, pulverized with alcohol, and then proceeded with for the separation of the albuminous compounds from the soluble constituents, as in the first quantity. The weight of the insoluble residue (*c*), minus the weight of its salts, corresponds to that of the albumin and hæmoglobin of the whole blood.

4. The fourth quantity is allowed to coagulate in a capsule. The serum is then poured off, and the albumin contained in a weighed quantity determined by the method already described.

The results stand as follows:—From 3, we learned the proportion in a known weight of blood, of albumin and hæmoglobin contained in the corpuscles; from 1, the corresponding proportion of albumin and hæmoglobin contained in the corpuscles and plasma together; and hence, by deducting the former from the latter, the proportion of albumin in the plasma. From 4, the proportion of albumin contained in the serum is known, and thereby that of the serum in the blood. The weight of the plasma is equal to the weight of the fibrin (2), *plus* that of the serum. Finally, by deducting the weight of the plasma from that of the blood, we have that of the corpuscles in the moist

24. Quantitative Determination of the Hæmoglobin contained in Blood.—It is often of great importance to be able to determine the proportion of hæmoglobin in a small quantity of blood; such, for example, as may be obtained by cupping. This is accomplished by making a solution of a measured or weighed quantity of blood in water, and then ascertaining, with the aid of the spectroscope, what degree of dilution is necessary in order to bring it to such a strength that only the red rays are transmitted (*see* § 18). The point of dilution at which the green is entirely extinguished, has been found by Preyer to be so constant, that it may be used as a basis for quantitative determinations.

The determination of the percentage of hæmoglobin which is required to yield the spectroscopic result above described, is accomplished by introducing a concentrated solution of a known weight of pure hæmoglobin crystals into a glass chamber (so-called hæmatinometer), of which the parallel sides are one centimeter from each other. The chamber is then placed in front of the slit of the spectroscope, the source of light being a paraffin lamp. Distilled water is then carefully added from a finely divided burette, so long as all of the spectrum is extinguished excepting the red. The moment that the green begins to appear, the operation is ended. The volume of the diluted solution is determined; and the exact conditions, viz., the distance of the lamp and chamber, and the width of the slit, are carefully noted. The percentage of hæmoglobin contained in the solution is that at which, *under the given conditions*, complete absorption of the green takes place. It may be designated . . k.

In order to ascertain the percentage of hæmoglobin contained in any given specimen of blood, all that is required is to repeat the process just described. A small quantity of fresh blood, which has been well agitated with air and defibrinated, is introduced into a finely graduated small pipette, from which exactly one centimeter is delivered into the glass chamber above mentioned, and diluted before the slit of the spectroscope (the liquid being carefully stirred after each addition) until the green begins to appear. At this moment the liquid contains a percentage of hæmoglobin equal to k. If the volume of distilled water including the centimeter originally added, be designated c, and the original volume of blood b, the percentage of hæmoglobin which the blood contains is readily calculated according to the formula

$\frac{x}{k} = \frac{b+c}{b}$ Whence, if the quantity of blood used, as above supposed, be one centimeter, we have $x = k\ (1 + c)$.

25. Determination of the Quantity of Hæmoglobin in

Blood, by the Estimation of its Iron.—Assuming that hæmoglobin contains 0·42 per cent. of iron, and that the whole of the iron of the blood is contained in its colouring matter, it is evident that if the percentage of iron existing in any quantity of blood is known, the percentage of hæmoglobin can be readily calculated. Although the process has disadvantages as compared with that last described, both as regards the time required for carrying it out, and the accuracy of the results, it cannot be omitted, as, under many circumstances (*e.g.*, when the blood to be investigated is not perfectly fresh), the spectroscopic method is inapplicable. To ascertain the proportion of iron in blood, a weighed or measured quantity of the liquid must be incinerated. The ash must then be dissolved in pure dilute hydrochloric acid, and the iron determined volumetrically with permanganate of potash. This is accomplished as follows :—

The volumetrical solution of permanganate which is usually employed, is prepared by dissolving the pure crystals in distilled water, in the proportion of 3·16 grammes to the litre. It is of such strength that 17·85 centimeters correspond approximatively to one-tenth of a gramme of metallic iron. It is, however, necessary, before using it, to determine its exact strength, by means of a weighed quantity of solution of the double sulphate of iron and ammonia. The mode of preparing this salt will be found in Sutton's "Volumetrical Analysis." It contains exactly one-seventh of its weight of iron, so that 0·7 gramme represents 0·1 gramme of iron. The mode of applying it is as follows :—

0·7 gramme of the salt having been dissolved in a beaker in distilled water, and five or six c. c. of dilute (1 : 5) sulphuric acid added, the permanganate solution is delivered from a burette, having a glass stopcock, until a point is reached at which the rose colour no longer disappears on shaking. As the permanganate must be slightly *in excess* to produce a perceptible colour, a correction should be made by ascertaining experimentally how much of the salt is required to produce the observed intensity of colour in the quantity of liquid used. This quantity should then be deducted from the result. The number of cubic centimeters used for 0·7 gramme of the double sulphate, (*i.e.*, 0·1 gramme of metallic iron) must be marked on the bottle. As the method depends on the conversion of the iron from the lower to the higher stage of oxidation at the expense of the permanganate, it is obviously necessary that the whole of the iron in the liquid to be operated upon should be in the condition (to use modern language) of a ferrous salt. For this reason, the first step in dealing with the hydrochloric acid solution of blood ash, is to reduce it. With this view, the solution of ash is first introduced into the flask already mentioned, in which it is gently boiled with a few pieces of zinc until the latter is dissolved and the liquid is colourless. It is then allowed to cool and diluted to fifty centimeters, after which the solution of permanganate is added to it from the burette, as before, until the rose colour becomes permanent after agitation. For each centimeter of the red liquid employed in attaining this result, the quantity of solution in the flask contains 0·0056 gramme of iron.

Section IV.—Gases of the Blood.

1. The gases of the blood are oxygen, carbonic acid, and nitrogen. The knowledge we possess of the conditions under which they are contained in the blood, and of the relative quantities of each, is founded entirely on the researches of Ludwig and his pupils, published during the first year of the last decade.

As regards oxygen, a correct method (that of displacement by carbonic oxide) had already been employed by Claude Bernard; but, as regards carbonic acid, the methods previously used were imperfect and the results erroneous.

2. In round numbers, one hundred volumes of arterial blood deliver to the Torricellian vacuum about twenty volumes of oxygen (estimated at 760 millimeters pressure and 0° temperature)—venous blood about twelve volumes. Of the quantity of oxygen so extracted, by far the greatest part is in combination with hæmoglobin—in other words, in the concrete state. The proportion of free oxygen in blood is so small that oxygen is absorbed from any atmosphere containing it in which its tension is greater than from twenty to twenty-five millimeters—in other words, from any space in which it exists in a proportion greater than about one-eighth of the proportion in which it exists in the atmosphere. Consequently, in subjecting blood to the air-pump, no oxygen is given off till the pressure sinks to about 125 millimeters (*i.e.*, about a sixth of an atmosphere); whereas, in the case of other liquids (*e.g.*, water), oxygen, with the other contained gases, begins to be disengaged, *pari passu,* with the reduction of pressure, in a quantity determinable according to Dalton's law. These facts are expressed by saying (1) that the absorption of oxygen by the blood is independent of Dalton's law, and (2) that the tension of oxygen in the blood is from twenty to twenty-five millimeters of mercury.

3. When blood is subjected to the Torricellian vacuum, the disengagement of oxygen is complete. The blood is converted into froth, and rapidly assumes a dark colour. This appear-

ance is due partly to the discharge of the colouring matter from the corpuscles, partly to the complete reduction of the hæmoglobin which accompanies the extraction from the *liquor sanguinis*, of its free oxygen.

4. When blood is subjected to an atmosphere which contains no oxygen, the result, so far as relates to the extraction of oxygen, is the same as if it were exposed to the vacuum. This is particularly the case if the gas employed be one which has the power of combining with hæmoglobin. The gas which pre-eminently enjoys this faculty is carbonic oxide. When blood is subjected to an atmosphere of this gas, the oxygen it contains, whether free or combined, escapes from it, its place being taken by carbonic oxide. The blood-colouring matter in combination with this gas acquires optical and other characters which remarkably resemble those of oxyhæmoglobin.

5. Carbonic acid gas may be extracted from arterial blood by the Torricellian vacuum in the proportion of about 35 volumes (as estimated at 760 millimeters pressure and 0° temperature) to 100 volumes of blood. Venous blood may yield 43 volumes, asphyxial blood 50 volumes. Of this quantity a certain but very varying proportion is merely absorbed, the rest is in loose combination, principally with the sodic carbonates of the plasma. It is probable that some of it is held by the bibasic sodic phosphate of the blood, and perhaps some otherwise. Hence it may be readily understood that serum contains as much carbonic acid gas as a corresponding volume of blood.

6. When a fixed acid, *e.g.*, tartaric acid, is added *in vacuo* to blood which has been already deprived of its absorbed and loosely combined carbonic acid (which together constitute what may be called its exhaustible carbonic acid), an additional quantity of carbonic acid may be obtained from it, which previously existed in the blood in the condition of neutral carbonate, principally if not entirely sodic.

Every apparatus for extracting the gases of the blood must consist of two parts, a mercurial pump and a recipient. The form and character of the latter necessarily depend upon those of the former. The most important forms of pump in use are those of Dr. Geissler, and others similar, employed in Germany, and of M. Alvergniat, in Paris. In this country, under the direction of Professor Frankland, Mr. Cetti has constructed a Sprengel's pump for the purposes of extracting the gases of water. Dr. Gamgee, of Edinburgh, has applied this form of pump to the extraction of the gases of the blood with complete success.

26. **Alvergniat's Pump.**—A long barometer tube, the scale

of which is divided into millimeters, is fixed to a vertical board on a suitable stand. This tube is dilated at the top into a large bulb (*a*, Fig. 197), and is then continued upwards until it ends in a three-way stopcock (*d*), surmounted by a funnel. To the right, the stopcock is in communication with a glass tube, ending in a bulb (*g*), and possessing a flexible joint at *f*. To the lower end of the barometer tube is fitted a long tube of thick-walled vulcanized caoutchouc, which ends in a globular mercury-holder (*v*). The vertical board is fitted at regular intervals with perforated shelves, on one of which the mercury-holder is resting. The pump is worked as follows:—*v* having been filled with mercury, the metal enters the vulcanite tube, and rises to the same height in the tube *a c* as in *v*. If *v* is raised from its present level to that of the highest of the shelves, the stopcock being at the same time turned so that the vertical tube communicates with the external air, but not with the bulb, the mercury will rise till the whole of the vertical tube is occupied. The stopcock is now turned so as to make communication only between *a c* and the bulb, and the mercury-holder is replaced in its original position. As the result of this manipulation, the air previously contained in the bulb and the tube leading from it, occupies the whole cavity, and (according to Marriotte's law) is expanded, *i.e.*, diminished in density in the same ratio that the volume occupied by it is increased. In other words, the density of the air in the bulb, before the depression of *v*, is to its density after, as the capacity of the barometer *plus* the bulb is to that of the bulb alone. To repeat the operation, the stopcock must first be placed in such a position that all channels are closed. *v* is then raised and the stopcock again turned as at first—viz., the horizontal way closed, the vertical way open. The air contained in *a c* having been discharged, the stopcock is again opened horizontally and closed vertically, and *v* depressed. The air remaining in the bulb is again expanded in the same proportion as before. If the capacity of the tube together with its dilatation be equal to that of the bulb and its tube, it is obvious that the effect of each stroke of the pump will be to halve the density of the air in the bulb; consequently, if the operation is repeated ten times, the density of the air contained in the bulb (supposing it to be dry, and to have an original density of 760 millimeters) becomes $760 \times (\frac{1}{2})^{10} = 0{\cdot}74$ millimeter. By filling the bulb and the tube leading to it, before attaching it, with water deprived of its gases by boiling, the process of exhaustion can be very much shortened. No sooner does the mercury sink in the vertical tube (*a c*) than the water follows it, and can be discharged by raising the mercury-holder with the stopcock open vertically and closed horizontally, as before. A vacuum which is almost perfect is thus obtained at a single working of the pump. In the pumps recently made by M. Alvergniat, he has substituted a movable support which works up and down the vertical board by a winch.

27. **Geissler's Pump.**—The instrument (*see* fig. 198) consists, like that just described, of a fixed vertical tube (*a*), which is dilated into a large bulb near the top and communicates near its lower end by means of a flexible tube of thick walled caoutchouc with another vessel (*b*) which can be moved up and down by turning a winch. Above the bulb, the vertical tube, which is nearly a meter in length, ends in a stopcock (*g*), so constructed that the bulb can be completely shut off, or may be brought into communication either with the external air or with the cavity to be exhausted. The pump is worked in the same manner as that just described. In order, if necessary, to dry the vacuum, a Pflüger's drying apparatus is interposed between the pump and the recipient. This may be described as a U tube, the bend of which is dilated into a bulb (*c*). It is so constructed that the fragments of pumice or the glass balls moistened with sulphuric acid which are used for drying can be readily introduced into either limb. The tube leading from the dessicator to the pump communicates with a vacuum gauge (*m*). The advantage which this instrument possesses consists in the relatively large size of the bulb, the perfection of the workmanship (particularly of the stopcocks) and the arrangement whereby the vacuum obtained is dry.

28. **Frankland-Sprengel Pump.**—Sprengel's pump, as modified by Frankland, consists essentially of a vertical glass tube (o Fig. 199) about four feet long, with thick walls and narrow bore, the lower end of which is bent up in such a way that, if filled with mercury, and closed at the top, it would constitute a barometer. At its upper end, however, it is not closed, but is continuous by a bend with the second vertical tube (G) or ascending limb of the Sprengel (the supply tube), which is of wider bore, and runs parallel to the first. At the top, or convexity of the bend, a third tube, about four inches in length (the exhaustion tube), is sealed on, by which the barometer tube or descending limb communicates with the cavity to be exhausted. The ascending limb communicates by a flexible tube, strengthened by a covering of strong canvas and guarded by a screw clip, with the descending limb of another bent tube (C) of similar construction to the first; the only difference between it and the one just described being that it communicates at the bend, not with any cavity, but merely with a bulb (D) closed at E by mercury. Its other limb finally communicates by a second flexible tube with a reservoir of mercury (B), the arrangement of which will be best understood from the figure. It consists of two glass funnels, each having long stems, the

relative sizes of which are such that the one can be contained within the other. To work the pump, the exhausting tube of the first bent tube must be connected with the cavity to be exhausted by means of a junction of vulcanized caoutchouc, guarded by a chamber filled with glycerin. Mercury is then poured into the inner funnel (the tube leading to the first bend having been previously closed) until it rises in the space between it and the outer to the same level. This done, the clip is opened, and a stream of mercury is allowed to flow over the two bends in succession, great care being taken that the stream is not so abundant as to cause the mercury to ascend in the exhausting tube above the level of the bend. The flow must then be gradually diminished with the aid of the clip, until the column of mercury in the descending limb of the Sprengel tube is broken into fragments by intervening spaces containing air. This happens whenever the quantity of mercury which reaches the bend by the ascending limb in any given time, is less than that which leaves it by the descending limb. In a time which varies according to the capacity of the cavity to be exhausted, vacuum is attained. No more bubbles are discharged at the lower end of the Sprengel. Each drop of mercury as it falls produces a peculiar click, and if the current is stopped, it is seen that the height of the column in the descending limb is less than that of the barometer at the time, by a number of millimeters which is equal to the tension of aqueous vapour at the temperature. The apparatus is so arranged that the bend of the first tube is supported at a level several inches higher than that of the second. Consequently, as the process of exhaustion approaches, the bulb with which it communicates becomes emptied of mercury, the vacuous space thus formed gradually extending till the level of the mercury in the descending limb coincides with that of the bend of the second tube.

We next pass to the description of the method of obtaining blood from an artery or vein, and of transferring it to the vacuum. Although it is not possible to produce a vacuum with the Sprengel pump above described, as rapidly as with the ordinary mercurial pump,* its action in other respects is very satisfactory. It completely fulfils the conditions enumerated by Ludwig as essential to an efficient blood-pump. The vacuum produced is perfect; it is bounded by mercury which, having previously passed through a vacuum (in the first tube), is completely deprived of air; and it can be renewed any number of times after the blood is introduced.

29. **Method of Transferring the Blood to be Exhausted from the Artery or Vein to the Vacuum.**—It is essential that the

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* The instrument probably admits of considerable improvement in this respect.

transference should be effected without contact with air; the blood must therefore either flow as directly as possible from the artery or vein into the vacuum tube; or, if it is intended to defibrinate it, it must be received in a space previously occupied by mercury. Before describing the mode of transferring, an account must be given of the chamber or recipient in which the blood is exhausted, and of the mode in which it communicates with the pump. The exhaustion tube (see Fig. 199, H) is connected by a vulcanite union, enclosed in an external tube containing glycerin, with a long nearly capillary tube, of such form and length as to reach the table by the side of which the pump stands. Near its lower end it is bent at an obtuse angle, so that the last few inches are horizontal. A little above the bend there is a bulb: the horizontal part is firmly supported on a block. With this tube the recipient is united either by a mercurial joint (I) or by a connector of vulcanized india-rubber, enclosed in a glycerin chamber. The recipient is a large glass tube (J), of about an inch and a quarter diameter, and forty inches long. At its lower end it terminates in a capillary tube, which is guarded by a stopcock (L). Its capacity is about 250 centimeters, consequently sixteen times that of the blood it is intended to receive.

In selecting a method of transference, preference ought to be given to those plans which are least complicated and most rapid in execution. The method I have found to answer is as follows:—The animal having been secured, a cannula fitted with an india-rubber connector is inserted in the vessel, which is closed by a clip *lege artis*. For receiving the blood as it flows from the artery or vein, a straight-glass tube (Fig. 199, M) of known capacity is used; one end of this tube is guarded by a stopcock, while the other is drawn out, and so formed that it can be accurately stopped by the finger. A trough having been filled with mercury, completely freed from air by passing through the pump, the narrow end of the tube is dipped into it. The tube is then easily filled up to the stopcock by aspiration and the stopcock closed. It having been ascertained that the tube is perfectly full, it is placed in an inclined position, with the stopcock end downwards, and the open end at such a distance from the cannula that the india-rubber tube can be easily slipped over it at the required moment. This having been accomplished, and the other end of the tube having been fitted with a bit of india-rubber tubing of sufficient length to convey away the mercury to a convenient receptacle, all is ready. The clip on the cannula is opened, and blood allowed to flow freely from the tube for a few moments while the mercury tube is grasped by the operator. The warmth of the hand causes the mercury to expand and project from the open end of the tube: at that moment the india-rubber connector from which blood is flowing is slipped over it, and the connection is completed without the slightest risk of the introduction of air. Without a moment's loss of time the stopcock is opened, and the blood allowed to replace the mercury. The stopcock having been closed, the india-rubber connector is slipped off, and the open end of the tube closed with the finger. The tube is now placed with its open end downwards in the mercurial trough (U), the finger being still kept on the orifice, while an assistant fills the bit of capillary tube beyond the stopcock with boiled distilled water, and connects it with the corresponding end of the recipient by means of an india-rubber connector. The moment that this is accomplished, the finger is removed from the orifice of the tube, and both stopcocks are opened. The blood passes rapidly into the recipient, followed by a column of mercury, and is at once converted into froth. A few drops of mercury having been allowed to enter, the stopcocks are finally closed. It will be understood from the figure that the joint between the measuring tube and the recipient, as well as the stopcocks, are under water, the purpose of which arrangement is, it need scarcely be said, to obviate the risk of the entrance of air.

At first the water in the wooden trough (N, which is not introduced until M has been joined to L) is kept cool with fragments of ice, in order to prevent the blood from coagulating during the preliminary operations. As soon as all is complete, hot water is gradually added until the temperature rises to about 40° C., care being taken not to expose the stopcocks to the air during the process. The only moment in the process at which air can be admitted, is that of joining the measuring tube to the recipient. For this reason it is desirable before opening the second stopcock of the measuring tube, to keep the pump in action for a few minutes so as to be certain that the vacuum is unimpaired before admitting the blood. This is not attended with inconvenience, if the blood is kept at a temperature approaching that of freezing.

When it is desired to defibrinate the blood before exhausting it, it must be collected over mercury. This is best effected in Ludwig's recipient. This recipient is a tube closed at one end and furnished with a Geissler's stopcock having a remarkably large way. The tube is inverted over mercury, with the stop-cock open, and the blood allowed to flow directly from the vessel into it until it is nearly filled. It is then closed by the hand, defibrinated by vigorous shaking with mercury, and replaced in the trough. The stopcock is now closed, and the tube, from which the blood contained outside of the stopcock has been washed, is united with the recipient of the pump by an india-rubber joint. To carry out this method, Sprengel's pump is scarcely applicable; for, inasmuch as the process of exhaustion cannot be begun until the connection is made, a long time must elapse before the tap can be opened. Blood alters so rapidly after removal from the body—the oxygen diminishing, the carbonic acid increasing—that if much time is lost the results are of little value.

30. **Method of Analysis.**—In France most of the analyses which have been published by Bernard and his pupils have been made by a method which, although rapid, is inexact. In Germany the analyses of Ludwig and his pupils, as well as those of Pflüger, have been made according to the accurate methods first introduced by Bunsen, and commonly known by his name. Bernard's method is practised in the physiological laboratory of the Jardin des Plantes, in Paris. The analysis is made in a circular mercurial trough, in the centre of which is a well sixteen inches deep, and large enough to contain about 12lbs. of mercury. The gas having been transferred from the tube in which it is collected from the pump, to a eudiometer, the latter is plunged into the mercury, in order that its contained air may acquire the temperature of the metal. It is then raised with the aid of a wooden tube-holder until the level of the mercury inside is the same as that outside. The quantity of gas having been measured, a fragment of caustic potash is introduced, which rapidly dissolves in the few drops of water which always float on the surface of the mercury. The column of mercury is then gently agitated by alternately raising and lowering the eudiometer, which, after the completion of absorption, is again plunged into the mercury. The gas having been again measured, about a centimeter of strong solution of pyrogallic acid is introduced with the aid of a pipette with a bent beak. The agitation is repeated and continued for some time. As soon as the absorption of the oxygen appears to be complete, the tube is transferred to a basin containing water, into which the mercury with the pyrogallate of potash is allowed to fall. The residue, consisting of nitrogen, is read over water. The results obtained by this rough-and-ready method must necessarily be erroneous, not only because the measurements are inaccurate, but because the absorptions must always be incomplete. If, however (as in certain pathological inquiries), it is more important that the analyses should be numerous than that they should be exact, it may be available. For class illustrations of the general nature of the blood gases it is completely adapted.

For more exact purposes the process of gas analysis has been during the last few years much shortened by Frankland, Russell and others. With a view to the analysis of the gases of drinking water, Frankland has introduced an apparatus of great simplicity (*see* Fig. 200), the working of which will be readily understood by the diagram. It consists of two parts, viz., a laboratory tube (*k*), in which the gas to be analyzed is first received, and a measuring apparatus, to which it can be transferred from the laboratory, in order that its volume may be determined before and after each absorption. The measuring apparatus consists of two tubes (*a*, *b*), fixed vertically side by side in a stand, surrounded by a chamber containing water (*n*). They communicate below both with each other and (by the long flexible tube) with a mercury-holder (*t*), like that of Alvergniat's pump. One of them can be brought into communication by the arm (*g*) with the laboratory tube; the other (*b*) is open at the top. A scale of millimeters is engraved on it, the zero of which is opposite *o*. A corresponding scale, starting from a zero at the same level, is engraved on the measuring tube. The apparatus is filled with mercury by raising the mercury-holder (*t*) to a sufficient height, the stopcock (*f*) remaining open; in doing which the surface of the mercury in *t* must not be more than a few millimeters higher than the tap. As soon as mercury appears at *g*, the stopcock is closed. The next step is to fill the laboratory tube. Having inverted it in the trough, which has been previously raised to the proper height, the operator draws out most of the air by means of a bent tube, the point of which rises to the top of the laboratory tube, and shuts the stopcock as soon as the mercury rises. The removal of the air is completed by joining *g* and *g′* so as to connect the laboratory tube with the measuring apparatus, and then causing the air contained in the former to pass over into the latter, by depressing *t*. The stopcock *h* must now be closed and *g* and *g′* disconnected to allow of the expulsion of the air from *a*. This having been accomplished, *g* and *g′* are again brought together and carefully secured. The whole apparatus is now full of mercury; as soon as it has been ascertained that the joint is air-tight at all pressures, it is ready for use. Before proceeding further, however, the measuring tube, which, as already stated, is graduated in millimeters measured from an arbitrary zero line near the bottom, must be calibrated. In other words, it must be ascertained as regards each principal mark of the graduation, what volume of air or water (as the case may be) the tube contains, when the upper convex surface of the mercury stands exactly level with it. For this purpose the orifice *a* is connected by means of an india-rubber tube with a reservoir (a funnel) containing distilled water. The mercurial column is then allowed to descend until it stands exactly at zero. A weighed beaker having been then placed under *a*, water is expelled till the column stands at a height of fifty millimeters, and the beaker again weighed. In a similar manner the outflow of water corresponding to a rise of the mercurial column from fifty to one hundred millimeters is determined, until the capacity which corresponds to each fifty millimeters of the scale is ascertained. To ensure accuracy, the process must be repeated several times. If the results, after correction for difference of temperature, are in close accordance, the means may then be taken as expressing the capacities required. In the upper part of the tube, calibration must be made at shorter intervals. In calibrating, as in all subsequent measurements, the height of the column must be read horizontally through a telescope, so adjusted that its axis is at the same height as the surface of the mercury. The temperature is read by a thermometer suspended in the cylinder of water by which the barometer and measuring tube are surrounded.

31. **Introduction of the Gas to be Analysed.**—The measuring and laboratory tubes having been brought into connection in the manner described above, and both filled with mercury, the gas to be analysed is introduced into the laboratory tube from the test tube to which it has been discharged by the Sprengel. It is then at once transferred to the measuring tube by depressing *t* until the mercury rises in the laboratory tube as far as the stopcock *g'*. This done, the stopcock *g* is closed, and *t* raised or depressed till the column stands at one of the marks of the graduation, in reference to which the capacity of the tube has been determined. The temperature is then observed, and the pressure determined by adding the difference between the height of the column in the measuring tube and that in the pressure tube, to the reading of a barometer which stands by. A few drops of solution of caustic potash having been introduced into the laboratory tube, the gas is returned from the measuring tube. Absorption takes place rapidly. It is accelerated by slightly agitating the trough, and by allowing the mercury to stream into the laboratory tube after the gas has passed. The measurement of the gas after absorption is performed in the same manner as before. About half a centimeter of strong solution of pyrogallic acid is then introduced in the same way as the potash, and the gas again returned. After absorption of the oxygen, what remains is nitrogen. In analysis of blood gases, the proportion of nitrogen is nearly constant, viz., about 2·5 volumes in 100 volumes of blood. If a larger quantity is obtained, the fact indicates that air has entered. Whatever method of analysis is employed, the results must be reduced to 0° temperature and 760° millimeters pressure—*i.e.*, they must be expressed as if the measurements

had been made under those conditions. A further deduction must be made from each measurement in respect of the aqueous vapour which the gas contains (the measuring tube being always moist). This is accomplished by the following well-known formula :—

$$V = \frac{V'}{1 + t\,0{\cdot}00367} \cdot \frac{H' - f}{760}$$

V denotes the corrected volume ; V' the volume read ; t the temperature ; H' the observed pressure ; and f the tension of aqueous vapour at the temperature t. The values of $1 + t\,0{\cdot}00367$ and f are always obtained from tables. For these, and many other important practical details relating to the performance of gas analysis, the reader is referred to Mr. Sutton's "Volumetrical Analysis," whom I have to thank for two of the woodcuts with which this section is illustrated. To illustrate the application of the method to the analysis of the gases of the blood, I give the following example :—

ANALYSIS OF GASES OF ARTERIAL BLOOD OF DOG.

	1st Measurement Total Quantity of Gas Extracted.	2nd Measurement. After Absorption of Carbonic Acid Gas.	3rd Measurement. After Absorption of Oxygen.
Height of Column in Measuring-tube	230·0	270·0	450·0
,, ,, Pressure-tube	312·8	369·0	320·0
Difference	82·8	99·0	—130·0
Reading of Barometer	764·0	764·0	764·0
H′=	846·8	863·0	634·0
Temperature=19·8° C.=t.			
Tension of Aqueous Vapours from table=f=	17·2	17·2	17·2
H′−f=	829·6	845·8	626·8
Volume of Gas as measured in cubic centimeters=V′=	11·822	3·865	0·562

$$1 + t\,0{\cdot}00367 \text{ (from table)} = 1{\cdot}0725.$$

Hence from first measurement we have—

$$V = \frac{11{\cdot}822}{1{\cdot}0725} \cdot \frac{829{\cdot}6}{760} = 12{\cdot}030.$$

From second measurement—

$$V = \frac{3{\cdot}865}{1{\cdot}0725} \cdot \frac{845{\cdot}8}{760} = 4{\cdot}010$$

From third measurement—

$$V = \frac{0{\cdot}562}{1{\cdot}0725} \cdot \frac{626{\cdot}8}{760} = 0{\cdot}432.$$

Thus the total volume of gases obtained as measured at 0° C. and 760 m. m. was 12·030 cubic centimeters : of carbonic acid gas was $12{\cdot}030 - 4{\cdot}010 = 8{\cdot}02$ c. c.: of oxygen $4{\cdot}010 - 0{\cdot}432 = 3{\cdot}578$ c. c. and of nitrogen 0·432 c. c.

As the volume of blood employed was 20·266 cubic centimeters, we have the following final result :—

In 100 volumes of blood—

Carbonic acid gas	39·585 volumes . .	$\left(=\frac{8{\cdot}020}{0{\cdot}20266}\text{ vols.}\right)$
Oxygen	17·652 . . .	$\left(=\frac{3{\cdot}578}{0{\cdot}20266}\text{ vols.}\right)$
Nitrogen	2·138 . . .	$\left(=\frac{0{\cdot}432}{0{\cdot}20266}\text{ vols.}\right)$
Total	59·375 . . .	$\left(=\frac{12{\cdot}030}{0{\cdot}20266}\text{ vols.}\right)$

In the preceding example such variations of temperature and barometric pressure as may occur during the analysis are disregarded. The readings are taken immediately after the absorption of the carbonic acid gas; as the time occupied in the analysis up to this point is very short, the error arising from the variations in question is inconsiderable. As regards the absorption of oxygen, the error might be of more consequence, were it not that the residue of nitrogen is so small. As it is, it can be easily shown that it would require a difference of pressure amounting to three millimeters, and a difference of a degree of temperature, to make an error of one-hundredth of a percentage in the result as regards nitrogen or oxygen. Within these limits, therefore, the errors arising from this source may be regarded as trivial.

Although determinations of oxygen made by absorption with hydrate of potash and pyrogallic acid are not entirely free from objection on the score of accuracy, the results obtained by the method above described are quite accurate enough for most of the purposes of physiological research, for the small errors are practically inappreciable, as compared with the variations in the proportion of oxygen contained in the blood to be analysed, produced by what might be regarded as very trifling differences in the mode of collecting it. If it is desired to have recourse to explosion with hydrogen, the best methods for the purpose are those of Dr. W. Russell and of Frankland and Ward. The following short description of the latter will be readily understood from what has preceded. The apparatus (Fig. 201) consists of two parts, corresponding to the laboratory-tube and measuring-tube of the instrument previously described. The measuring-tube communicates, as in that instrument, with a second tube (the one most to the right in the figure) containing a column of mercury, by the height of which the pressure to which the gas to be measured is subjected, can be estimated. The chief difference is that, whereas in the former more simple instrument the pressure-tube is open at the top, so that if air is contained in the measuring-tube, and the stopcock by which it communicates with the laboratory-tube is closed, the difference between the heights of the two columns indicates the difference between the tension of the gas in the measuring-tube and that of the atmosphere—in the instrument now before us the tube is closed and constitutes a barometer, so that the difference expresses the actual tension of the gas in inches of mercury. In the horizontal channel, by which the measuring-tube and barometer communicate at the bottom, is a three-way stopcock (not shown in the figure), by which they may be brought into communication either with a vertical escape-tube, the end of which dips into a receptacle containing mercury several feet below, or with a tube open at the top (the middle and longest in the figure), called the filling-tube. In this way the gas can be expanded or compressed at the will of the operator, and consequently can (in most analyses) be readily brought to the same volume after each successive operation. The convenience of this is very great, for obviously the tensions of different quantities of gas when expanded to the same volume are proportional to the volumes they would assume if they were all under the same pressure, so that the original volume of gas to be analysed being known, the relation between that volume and the volume of the other quantities to be measured can be readily calculated, the several volumes being proportional to the corresponding readings of the barometer. The original volume of gas to be analysed is measured as before described, with this difference, that the absolute pressure to which it is exposed is known without reference to the barometric pressure outside at the time. The explosion is effected in the eudiometer, into the upper end of which two platinum wires are fixed for the purpose; the arrangement of these wires is the same as in Bunsen's eudiometer. As to the mode of preparing and introducing pure hydrogen and of exploding the mixture, the reader will find sufficient information in Roscoe's translation of Bunsen's Gasometry.

32. **Bernard's Method of determining the Proportion of Oxygen combined with the Colouring Matter of the Blood by Displacement with Carbonic Oxide.**—As was before stated,

the property which carbonic oxide possesses of displacing the oxygen combined with the colouring matter of the blood, has been used by Bernard, as a substitute for the vacuum, for the determination of the quantity of free and combined oxygen contained in the blood. Bernard's method consists in agitating the blood to be analysed in a tube half filled with carbonic oxide. The carbonic oxide to be used must be perfectly pure. The tubulated retort into which the oxalic and sulphuric acid are introduced must be cleared of atmospheric air, by passing a stream of carbonic acid through it, before heat is applied. The gas is best collected in flasks, over water containing potash in solution. Two results are produced. In the first place, the oxygen of the hæmoglobin is replaced by carbonic oxide; and, secondly, the atmosphere of carbonic oxide acts on the blood as if it were a vacuum, the displaced oxygen and other gases passing out into it until equilibrium is established. Inasmuch as the proportion in which oxygen is absorbed is very small, as compared with the quantity held in combination by hæmoglobin, nearly the whole is discharged, so that if the proportion of that gas contained in the gaseous mixture which fills the place originally occupied by the carbonic oxide be determined, it is found to fall very little short of the proportion obtained from the same blood by exhaustion. The remainder of the mixture contains, in addition to the excess of carbonic oxide, nitrogen and carbonic acid gas, derived from the blood, but the proportions of these gases discharged are very variable. As regards oxygen, the method has yielded, in the hands of Bernard, results of the greatest value. It has the immense advantage that it can be carried out without a mercurial pump, and for pathological purposes is sufficiently accurate.

CHAPTER XVI.

THE CIRCULATION OF THE BLOOD.

In commencing the study of the circulation of the blood, it is desirable to direct our attention first to that part of the circulatory apparatus in which the phenomenon presents itself in its simplest form. In systematic physiological treatises the heart is usually described first; but for our present purpose, considering that the heart is an organ of very complicated structure, that it is constantly influenced by ever-varying conditions of the vessels on the one hand, and of the nervous centres on the other, it is much better to begin with the arterial system.

Part I.—The Arteries.

At the commencement of the period of relaxation of the heart—*i.e.*, of the period which intervenes between one contraction and its successor—the progressive movement of the blood in the aorta all but ceases. At that moment, and during the remainder of the time which precedes the bursting open of the aortic valve, the pressure exercised by the wall of the vessel on its contents is the only cause of the continuance of the blood-stream. During each ventricular systole the aortic pressure is reinforced by the motion communicated to the blood by the contracting ventricle. Consequently, if—for the sake of facilitating our understanding of the matter—we assume the heart to be a mere pump, acting regularly, and discharging at each stroke an invariable quantity of liquid, we have the force by which the circulation is carried on at any moment expressed by the tension of the arteries, and varying with that tension; or if, on the other hand, we assume the tension of the arterial system to remain constant, then the quantity of work done varies with the mean velocity of the stream at the commencement of the aorta—in other words, with the quantity of blood delivered by the heart per minute.

The work done by the heart in maintaining the circulation, manifests itself in the aorta in two modes, those of *pressure* and *progressive motion* of the blood. These two phenomena are not, however, collateral results, *i.e.*, they do not stand in the same relation to the agent which produces them. The former is rather the efficient cause of the latter; for so long as the arterial pressure continues, *i.e.*, so long as the pressure in the aorta is *greater* than that in the venæ cavæ, progressive movement also continues. As soon as equilibrium is established, circulation stops. Systemic death consists in decline of aortic pressure. This decline may occur rapidly, as in syncope; but usually, even in deaths by violence, it is very gradual. In deaths from disease it may last for days, weeks, or even months.

SECTION I.—ARTERIAL PRESSURE.

33. The arterial pressure, although in the mean remarkably constant, almost as constant as the temperature of the body, is subject to recurring variations—*i.e.*, alternate augmentations and diminutions, which are of three orders. Of these, the first is dependent on the rhythmical injection of blood into the arteries by the contraction of the heart; the second, on the influence which the respiratory movements, or rather the alternate acts of breathing, exercise on the circulation; the third, on augmentations or diminutions of what is called the *tonus* of the arteries, by virtue of which they are constantly undergoing changes of diameter, consequent on varying conditions of the nervous system.

In the measurement of the arterial pressure we have, therefore, two distinct problems. The first is the determination of the mean or average pressure, which, as I have said before, is almost as constant as the temperature in the same animal so long as it remains in a natural state; the second is the investigation of the variations due to the heart's action, to respiration, or to arterial contractility, respectively.

For the determination of the mean arterial pressure, and of those variations which belong to the second and third class, preference is to be given to the ordinary mercurial manometer, one branch of which is connected with the artery to be investigated, while the other is open. This instrument, as so applied, constitutes what Poiseuille designated by the term hæmadynamometer. It was employed in this simple form until Ludwig, in 1848, by his invention of the kymograph, laid the foundation of the more exact methods of investigating blood-pressure which are now in use. Just as the first method of Poiseuille originated in the ruder experiments of our countryman Hales, so the notion of the kymograph is said to have been suggested by a contrivance of Watt's for registering the pressure of the steam-engine.

The principle of the kymograph consists in causing a pen, fixed horizontally at the upper end of a vertical rod, the lower end of which rests by a floating piston on the surface of the mercurial column in the distal open limb of the manometer, to write the up and down movements of the column on a surface of paper progressing horizontally at a uniform rate by clock-work. Since the time that Ludwig first employed it, the contrivance has developed into a method now commonly known as the graphic method.

Description of the Kymograph and Accessory Apparatus now used in the Laboratory of University College.*—1. The arterial cannula is a ⊤-shaped tube of glass, of the size and form shown in fig. 193, *c*. By its stem it is connected with the manometer; one branch is drawn out and bevelled, the other is of the same size as the stem, and when in use is fitted with a short bit of caoutchouc tubing, guarded by a steel clip.

The cannulated end is made as follows:—The tube which it is intended to use for the purpose is first softened in the flame of the gas blow-pipe, and drawn out gently at the softened part. It is then allowed to cool, and again heated in a pointed flame at *x*, and drawn out so as to make it assume the form 193, *b*. It is then scratched with a sharp three-cornered file opposite *x*, and sundered by drawing the one end of the tube from the other in the direction of its axis. The last step in the process consists in filing off the cut end in the direction of the dotted line, and smoothing the edges by touching them with the border of an ordinary gas flame. A tube of this kind can be inserted with great ease into an artery of considerably less diameter than itself. Cannulæ of glass are always to be preferred to those of silver, not merely on the ground of facility of introduction, but because a glass surface is much less apt than one of metal to determine coagulation of the blood which comes into contact with it.

2. The stem of the arterial cannula communicates with the proximal arm of the manometer (*see* fig. 202) by a tube (*c*), of which the part next the cannula only is of india-rubber. The rest is of lead; the purpose of the arrangement being to avoid a certain modification of effect due to the yielding of the wall of the tube, which becomes appreciable if the whole connector is elastic.

3. The proximal arm of the manometer communicates at its end, by means of a long flexible tube (*b*) guarded by a clip, with a "pressure bottle" containing solution of bicarbonate of soda. A horizontal arm, which springs from it near the top, is continuous with the lead tube already mentioned.

4. The manometer is fixed to the edge of the small mahogany table on which the recording apparatus stands by means of brass clamp, which admits of its being raised or lowered at will.

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* This instrument was made for me by Mr. Hawksley, of Blenheim Street, and has advantages over any other form with which I am acquainted.

The floating piston and rod (*a*) are made of black vulcanite. The piston is in the form of an inverted cup, which embraces the convex surface of the mercurial column. The rod is quadrangular, and works in a guide, fixed at a height of six inches above the upper end of the tube, by which it is kept vertical. The writer, a fine sable miniature pencil, is supported on the rod by a horizontal arm of thin wire, one-third of an inch in length. One end of the wire is coiled round the rod, the other round the stem of the pencil. From the guide just mentioned springs a horizontal arm, from which a silk plummet-line is allowed to fall in such a way that it rests against the horizontal part of the wire. By this means the point of the writer is kept in constant contact with the paper, without exercising too much pressure.

6. The recording apparatus consists of a single cylinder, which revolves at a constant rate of one revolution per minute. The clock-work by which it is moved is constructed by Mr. Hawksley on the model of the so-called "Foucault's Regulator." To the right of the cylinder, as seen in the drawing, is shown a large brass bobbin, of the same width as the cylinder, on which a riband of paper is tightly rolled by machinery, of sufficient length to serve for many hundred observations. From the bobbin the paper riband is drawn off by the cylinder as it revolves, against the surface of which it is accurately applied by a spring, furnished with ivory friction wheels.

34. Rules and Precautions to be observed in making a Kymographic Observation.—Before commencing, it is necessary to see that the manometer is in proper order. The mercury in the distal column must be clean and dry, and the writing pencil moist and free from the remains of the ink. To ensure this, it should always be steeped in water after each observation.

To dry mercury, the best Swedish filtering paper is used. It is cleaned by straining it through calico, or still better through chamois leather. If the latter is used, it must be strained under a considerable pressure. The system of tubes communicating with the proximal limb of the manometer must now be filled with solution of bicarbonate of soda. To accomplish this, the arterial tube is first closed by a clip, and the solution introduced with the aid of a pipette into the open end of the proximal limb. Some of the solution is then allowed to flow from the bottle by the long communicating tube (*b*) so as to fill it completely, after which its end is brought into communication with the manometer. If any air bubbles are introduced, they are readily got rid of through the artery tube. According to the height to which the pressure bottle is raised above the level of the manometer, the mercurial column in the distal limb rises above that in the proximal. It must be adjusted so that the difference between the two is a *little* *less* than the probable arterial pressure of the animal to be used. This having been accomplished, and the *communication between the manometer and the pressure bottle closed,* all is ready.

The only arteries which are used for observations of arterial pressure are the carotid and the crural. On the whole, the latter is preferable; for the carotid cannot be exposed without some risk of disturbing the vagus nerve. In the rabbit, the carotid is prepared as follows:—The animal having been secured on *Czermak's rabbit-board,* and the fur clipped, the skin is pinched up between the finger and thumb on either side of the upper end of the trachea, so as to form a horizontal fold, which an assistant divides vertically. As soon as any slight bleeding has ceased, the wound is dabbed with a sponge moistened with saline solution, and the fascia, which stretches from the edge of the sterno-mastoid to the middle line, is seized with *blunt forceps* and opened with *knife or scissors.* The opening having been enlarged with the aid of a *second pair of blunt forceps,* the sterno-mastoid is slightly drawn aside, so as to bring the artery, with its three accompanying nerves, the vagus, the depressor and the sympathetic, into view. The sheath having been opened, the artery is raised on a *blunt hook,* and easily cleared from its attachments to a distance of three-quarters of an inch in either direction. The distal end of the prepared part is tied, and the proximal end closed by a *clip.* A *splinter of wood,* or a bit of card of similar shape, is slipped under the artery close to the *ligature,* and a second *ligature* looped round it. Finally a V-shaped snip is made in its wall with *scissors* which cut well at the point; the *cannula* is inserted, and the ligature tightened round the constriction. The whole operation ought to be accomplished in three minutes; it is desirable to have an assistant. The instruments required are indicated by the italics. (*See* fig. 203.) They must be placed in readiness on the table of the kymograph. Czermak's rabbit supporter is shown in fig. 204. It consists of a strong wooden board, about 8 inches wide and 30 inches long. At one end it is strengthened with an iron plate, into which a strong vertical stem is screwed. This stem bears a sliding block of brass, in which an iron rod also slides horizontally. Near its base it is bent twice at right angles, so that the upper part on which the block slides is not in the same line with the lower part. Consequently the rod, while still remaining horizontal, can be moved in four different ways. It can be shortened or lengthened, heightened or lowered, rotated round its own axis, rotated round the axis of the stem, or moved from side to side without change of direction. It ends in a kind of forceps the blades of which, when kept closed by the adjusting screw, seize upon the head of a cat or rabbit in such a manner as to hold it firmly without inflicting the slightest injury. The neck of the animal rests on a cylindrical cushion, covered with water-proof cloth, and the rest of the body on a mattress of similar material. Along the edges of the board there are convenient attachments for the extremities.

The preparation of the crural artery is even more simple than that of the carotid. The skin having been divided in a line leading from the middle of Poupart's ligament towards the inner side of the knee by first pinching up a fold of skin as above directed, the pulsation of the artery is felt by the finger in the hollow between the adductor muscles and those which cover the femur. The sheath of the vessels having been exposed from Poupart's ligament downwards, the vein and crural nerve are seen, the artery lying behind and to the outer side of the former. On drawing the vein inwards it is easily got at, and must be prepared from the origin of the *arteria profunda* close to Poupart's ligament, nearly to the point at which it enters the adductor; first giving off the *arteria saphena*, which accompanies the saphenous nerve and veins. The lower of the two circumflex arteries which are given off within a short distance from the profunda must be tied doubly and divided between the ligatures, as it is desirable to place the clip as high as possible. In the dog or cat, the operation is equally simple, but requires more time on account of the greater abundance of fat in these animals.

The cannula having been inserted, the next step is to bring the artery into communication with the manometer. The clip on the artery remaining closed, that on the stem of the cannula is opened for a couple of seconds. At once the soda solution fills the cannula and passes out by its open branch. In doing this, great care must be taken not to allow the solution to flow into the wound. Air bubbles, if they exist, are got rid of by passing a thin rod of whalebone into the cannula, which must then be closed by means of the terminal clip. All being now ready, the stem of the cannula is finally opened, and the clip removed from the artery. The mercurial column at once begins to oscillate; but no record should be taken until a minute or two have elapsed, for it often happens that a small quantity of soda solution enters the artery and produces a slight and transitory disturbance of the circulation. If, indeed, the previously existing pressure in the artery tube is somewhat less than that of the artery, no such effect occurs; but inasmuch as we have no means of knowing the arterial pressure of any particular animal beforehand, it is usually unavoidable.

A kymographic observation may last a few minutes or several hours, according to the question to be investigated. In the latter case, tracings are taken at intervals. Two persons are required, one of whom performs the experiment, while the other undertakes the charge of the writing apparatus, and notes on the paper-roll, with a soft pencil, the events as they occur and the times of beginning each tracing. In this way the roll stands in the place of a protocol, and is less liable to errors of time and order than any other kind of record.

35. Measurement of absolute Arterial Pressure at any given moment during the period of observation.—For this purpose it is necessary to draw the abscissa of the pressure curve, *i.e.*, the horizontal line which the writer would have drawn had the arterial pressure been equal to that of the atmosphere. This is accomplished immediately after the termination of the experiment, by closing the stem of the cannula and then removing it from the artery, and immersing it in a capsule containing soda solution, standing at a level equal to that of the artery. The clip having been opened, the clock-work is set in motion for a moment, and a horizontal line drawn which coincides with the abscissa required. In this line the paper is then pierced with a pointed instrument in such a way as to perforate the several layers of paper at the same level. By removing the roll from the cylinder and connecting the holes, a horizontal straight line is obtained which runs from end to end of the record. By drawing an ordinate from any point in the tracing to this line, measuring its length in millimeters and doubling the result, the absolute arterial pressure at the corresponding moment is obtained in millimeters of mercury.

The *mean arterial pressure* is obtained by drawing ordinates at regular intervals and measuring the length of each. The mean of the lengths corresponding to the period investigated, multiplied by two, is the mean pressure required. [I never use paper divided into squares—in other words, with the ordinates ready measured—finding by experience that they do not tend to accuracy. Moreover, such paper is expensive and thereby furnishes an inducement for an undesirable economy in its use.] In all normal kymographic records it is seen that the arterial expansions due to the contractions of the left ventricle are indicated by oscillations which differ very materially in form, and that these differences are dependent on their frequency. (*See* fig. 206.) When extremely frequent, they are mere undulations; but when the intervals are longer, they exhibit forms which, as we shall afterwards see, have a definite relation to the changes of tension which actually occur in the arteries during each cardiac period. It is further seen that there are larger waves which correspond, not to the beats of the heart, but to the respiration—the valley and ascending limb of each of these greater undulations corresponding to inspiration, the summit and descending limb to expiration and to the pause. These and other details will be referred to in future sections.

SECTION II.—OBSERVATION OF THE SUCCESSIVE CHANGES OF ARTERIAL TENSION WHICH OCCUR DURING EACH CARDIAC PERIOD.

In studying tracings obtained by the mercurial kymograph, it is to be borne in mind that what is inscribed on the cylinder is not the record of the actual movement of the artery, but of the oscillations of the mercurial column. It is true that the latter are the immediate results of the former, and that the elevation of the distal column produced by each arterial expansion has some relation to the increase of lateral pressure, of which the expansion is the expression; but the curve drawn is not that of the artery, but of the manometer. The artery expands suddenly, the mercury rises comparatively slowly, so that at the moment it attains its acme the artery has already collapsed. Consequently, if the interval between each pulsation and its successor is very short, the extent of oscillation (or, as it is usually called, the excursion) of the manometer is relatively too small; and conversely, if the interval is much prolonged, the excursion is relatively too great. The descent of the column is almost entirely independent of the collapse of the artery. It falls back to equilibrium, and describes a curve, which (as may be learnt by comparison) has the same characters as that made by the lever in returning to its original position, by whatever way—as, *e.g.*, by squeezing the connecting-tube—the equilibrium of the manometer may have been momentarily disturbed.

This being the case, it is easy to understand that no conclusion can be derived from observations with the mercurial manometer, either as to the duration of the effect produced by each contraction of the heart, or as to the relative duration of the periods of expansion and collapse. The use of the instrument is limited to the investigation of the mean pressure, and of those varieties of pressure of which the periods of recurrence are long enough to prevent their being interfered with by the *proper oscillations* of the instrument.

36. **The Spring Kymograph.**—If we desire to obtain a record of the complicated succession of variations of arterial pressure which constitute an act of pulsation, precisely as they occur as regards order, duration and degree, or of the exact interval of time between the close of one arterial expansion and the commencement of the next, the instrument with which we write must be of such a nature that it shall transmit the movements communicated to it without mixing with them any movements of its own. The most perfect of such instruments is the so-called *Federkymographion* of Professor Fick. The construction of the instrument will be readily understood with the aid of

Fig. 205. It consists essentially of a C-shaped hollow spring of thin metal. The cavity of the spring is filled with spirits of wine, and communicates with the artery by means of a connecting-tube containing bicarbonate of soda. As the pressure increases, the crescentic spring tends to straighten, and *vice versâ*. Hence, if the proximal end is fixed, the distal end performs movements which follow exactly the variations of arterial tension. These movements are of very small extent, but they are so exact that the slightest and most transitory variations are expressed by them. Before they are written on the cylinder they must be enlarged by a lever.

It is not necessary to make any remarks as to the mode of connecting the spring kymograph with an artery, the *modus operandi* being the same as that described in § 34. It is, however, to be noted, that if it is intended to use the tracing obtained by it for the purpose of determining the absolute arterial pressure, the instrument must be first graduated by comparison with a mercurial manometer. This is effected as follows:—The kymograph being placed so as to write on the recording cylinder, its artery tube, which communicates by a side opening with a pressure bottle, is united with the proximal arm of the manometer. The pressure bottle is first lowered until the liquid it contains stands at the same level as the mercury in the proximal arm. A tracing is made on the cylinder, which is the abscissa. The bottle is then raised till the distal mercurial column is ten millimeters higher than the proximal, and a second tracing taken, and so on at successive increments of 10 mill. pressure, up to 150 mill. or more. By measuring verticaly the distances in millimeters between the horizontal lines so traced and the abscissa, a series of results are obtained which express the values of the ordinates of the tracing in millimeters of mercurial pressure.

In tracings obtained by the spring kymograph it is seen that the ascent of the lever, which corresponds to the period during which the artery is acted on by the contracting ventricle, is abrupt—indeed, nearly vertical; that towards the vertex the tracing changes direction, gradually approaching a horizontal line touching it at the *highest* point; that the line of descent—much more oblique than that of ascent—terminates in the same way by gradually approaching a horizontal line touching the curve at its *lowest* point. (*See* fig. 207.)

37. Observation of the Expansive Movements which accompany the successive Changes of Arterial Pressure above described.—When an artery is exposed in a living animal, as, *e.g.*, when it is prepared in the manner described in § 34, two kinds of motion are seen. The bit of artery which is separated from the surrounding parts lengthens, and its diameter visibly increases each time it is acted on by the contracting heart.

Of these two phenomena, the first is commonly called locomotion, because in certain superficial arteries of the human body (especially when they are enlarged in advanced life), the artery, as it lengthens, is compelled to bend to one side or the other, and thereby visibly changes its place each time that it is distended. The other, viz., the expansive movement, is called *pulsation,* and is practically of great importance, seeing that it is the only phenomenon of the arterial circulation which admits of being investigated without exposing the artery, and consequently affords the only direct means by which we can judge of its ever-varying conditions in man.

Arteries being elastic, their changes of diameter express all changes of the pressure exercised by their liquid inelastic contents on their internal surfaces. If, therefore, the expansive movements of an exposed artery were to be measured and recorded graphically, the record would correspond closely with that of the pressure obtained by Fick's kymograph. For just as in that instrument the variations of pressure are converted by the C-shaped spring into nearly rectilinear movements, the artery expands with every increase of pressure on its internal surface, and contracts with every diminution of it; so that any point taken on its surface is constantly performing, in relation to its axis, orderly successions of rectilinear movements in opposite directions.

In both cases—that of the spring and that of the artery—the *expansion,* and the *pressure* which produces it, vary in the same directions during the same times, but *not in the same degree.* As regards the spring, we can readily determine the relation of expansion to pressure by the method of graduation described in the preceding paragraph, and so use the former as an expression for the latter. In the case of the artery, no such empirical graduation is possible. The expansion of an artery, or any other elastic tube, due to any given increase of pressure against its internal surface, depends upon the degree in which the tube is already distended at the commencement of the act of expansion. The greater the original distension, the less will be the effect; so that the condition of an artery in which the expansive movement is relatively greatest, is that in which its walls, when the expanding agency is suspended, are in the state of elastic equilibrium, *i.e.,* when the minimum pressure is least. A moment's consideration teaches us that there are two circumstances which must diminish the minimum pressure in the arteries, viz., diminution of the mean arterial pressure, and prolongation of the period which intervenes between one expansive act and its successor. In other words, *the less frequent the contractions of the heart and the lower the arterial pressure, the greater the expansion in proportion to the expanding force which produces it.*

38. The Sphygmograph.—In man, no artery can be directly measured either as regards pressure or expansion. In feeling the pulse, we attempt to measure both by the sense of touch, and obtain results which, although incapable of numerical expression, are sufficiently exact to be of great value. In the sphygmograph, an attempt has been made to obtain the same kind of information by a mechanical contrivance, which the physician obtains by the *tactus eruditus*; the supposed advantage of the instrumental results over the others being, that they can be estimated by measurement and weighing, and that they are unaffected by variation in the skill and tactile sensibility of the observer.

The purpose of the sphygmograph is to measure the complicated succession of alternate enlargements and diminutions which an artery undergoes whenever blood is forced into it by the contracting heart, to magnify those movements, and to write them on a surface, progressing at a uniform rate by watch-work.

The construction of the instrument is so well known, that it is scarcely necessary to give a detailed description of it. It consists essentially of three parts: a frame of brass which is applied along the outer edge of the volar aspect of the fore-arm, in such a way that it is maintained in a fixed position with reference to the bones of the wrist and radius—a steel spring which, when the instrument is in use, presses upon the radial artery and receives its movements—and lastly, mechanical arrangements for magnifying these movements and recording them. Both of these ends are accomplished by means of a light wooden lever (A A′, fig. 208) of the third order, which is supported by steel points (C). There is a second lever of the same order (B E) which has its centre of movement near the attachment of the spring (at E). It terminates in a vertical knife-edge (D), and is traversed by a vertical screw (T). When the extremity of the screw (N) rests upon the spring above the ivory plate, every movement of the plate is transmitted to this lever (B E), and, by means of the knife edge, to the wooden lever (A A′). The purpose of the screw (T) is to vary at will the distance between the wooden lever and the upper surface of the spring, without interfering with the mechanism by which the movement is transmitted. As the distance between the steel points (C) and the knife-edge (D) is much less than the length of the lever, the oscillations of the extremity of the lever (A′) are much more extensive than the vertical movements of the spring. The lever ends in a metal point, which writes on a glass plate blackened by passing it rapidly backward and forward through the flame of a spirit-lamp trimmed with paraffin.

When this instrument is applied in the proper manner to the wrist, the radial artery is compressed between the surface of the radius and a spring, the bearing of which is in a fixed position in relation to that surface. This being the case, the spring performs movements which are more or less conformable with the variations of the diameter of the artery. These movements are transferred in a magnified, but otherwise little altered, form to the lever. As regards the relative and actual duration of the movements, the correspondence is exact; but as regards their extent, this is true only in so far as the lever follows the movements of the spring with precision,* and as the strength of the spring, *i.e.*, the pressure exercised by it on the artery, is adapted to the antagonistic pressure exerted by the blood stream on the internal surface, and to the extent of the movements it is intended to measure.

The relation between the pressure of the spring and its effect on the artery is a complex one, and need only be considered here in so far as is necessary for the interpretation of sphygmographic results. To facilitate our understanding of it, let us call the position which the spring takes when left to itself its *equilibrium position;* and as regards the artery, let us designate a plane parallel to the surface of the skin and touching the surface of the artery, when most dilated, the *plane of expansion;* and a plane in similar relation to it, when least expanded, the *plane of collapse;* and to simplify the problem let us suppose that the artery is not covered by skin. It is evident that, if the sphygmograph accomplished its professed end completely, the under surface of its spring would coincide with one of these planes at the moment of the pulse, and with the other during the interval. The question is, How ought the spring to be set, in order to obtain a movement which shall approach this standard of perfection as nearly as possible? We may proceed one step towards answering this question without difficulty. It should be set so that if the spring were in the equilibrium position its under surface would lie within the plane of collapse—*i.e.*, nearer the axis of the artery. For if it were further from the artery it would be affected by the arterial movement only during its period of expansion, remaining the rest of the time motionless. If, on the other hand, it were much nearer, the vessel would be flattened against the bone during the period of collapse, so that in this case, as in the other, there would be no motion (of the spring) during diastole. Hence it is easy to understand how it happens that the tracings obtained with excessive and defective pressure are very similar to each other in their general characters. Stating the same thing in other words, we arrive at the general rule that the spring must be so set that the ivory plate on its under surface is at such a distance from the opposed surface of bone, that the artery is pressed upon at all degrees of expansion, yet not so strongly pressed upon as to bring its walls into contact even when it is relaxed. Within these limits, the variations of form of the tracing—in other words, its departure from truth—are very inconsiderable; so that observations made on the same individual at different times yield closely corresponding forms. As, however, the results obtained by strong pressure are less subject to accidental error than those obtained with weaker ones, it is better always to begin with a pressure sufficient to flatten the artery, and then to weaken the spring until the effects of over-compression disappear—*i.e.*, until it is found that the lever continues to descend until the very end of diastole.

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* See note on p. 226.

39. Use of the Sphygmograph as a Means of appreciating those Changes of Mean Arterial Pressure which occur in Disease.—We have already seen that the sphygmograph is of no use as a gauge of arterial pressure. It is possible, however, by the comparison of observations made at successive periods on the same individual, to determine whether the arterial tension has changed, and in what direction the change has taken place. We have seen that if the spring is so strong that the artery is either partially or entirely flattened against the radius, the fact is indicated by the cessation of the motion of the lever. The strength of spring which is required to bring about this result varies with the pressure by which the artery is distended; so that if in any individual the arterial pressure is increased, a greater tension of the spring is required to compress it than was required before. With Marey's sphygmograph, as imported, it is not possible for the observer to avail himself of this principle, because the instrument is not graduated, *i.e.*, there is no means by which the pressure exerted by the spring at any moment can be ascertained. I have therefore modified that instrument as follows (*see* fig. 209, *a*):—The brass frame, instead of being bound on to the arm by bandages, rests firmly on the bones of the wrist (particularly the scaphoid) by a plate of brass, the under surface of which is covered with ebonite. In the middle of the upper surface of this plate is a socket for the reception of the point of a finely-cut screw, which revolves in it freely. Above, the screw ends in a milled head (Y), between which and its point it passes, first loosely through a guide, which is of the same piece with the brass-plate; and, secondly, through a hole in the end of the brass frame of the sphygmograph (F), in which it fits closely. This being the construction, it is scarcely necessary to explain that by turning the milled head, the distance between the ebonite surface and the frame is varied according to the direction of revolution, and that in this way the pressure on the artery may be readily modified when the instrument is in use. The extent of the modifications thus produced, however, still remains undetermined, for they vary according to the form of the limb and the relative position of the arm and forearm at the time of observation. To measure them, we must have recourse to another method which is at once simple and accurate. It is obvious that, provided that the spring is firmly and immovably fixed in its place, the pressure which it makes against any object pushed against it from below is determinable by the force which is exerted in pushing it. If, for example, I turn the instrument upside down, and place a weight of 200 grammes on what was before the under surface, now the upper surface, of the spring, I push it back some fraction of an inch from its position of equilibrium; I learn that, whenever it is pushed back to this extent, the pressure it exerts on the surface opposed to it is that of 200 grammes' weight. Repeating the experiment with a series of other weights, I can in a similar way obtain other measurements of distance corresponding to them, and thus, by combining the results, accomplish the graduation of the spring in such a way that the pressure made by it can be always known from the extent of its deflexion. The most convenient way of determining this deflexion is either to measure the distance between the head of the steel screw (the point of which rests on the upper surface of the spring) and the surface of the brass lever, with a scale (as shown in fig. 210), or, better still, to have the screw itself graduated. In either case, care must be taken to fix the writing lever in the proper position (*i.e.*, in a direction which coincides with the direction of movement of the writing surface) before making the measurements.

40. **The Artificial Artery or Arterial Schema.**—The phenomena of arterial pulsation can be best studied in a well-constructed schema or artificial artery, consisting in an elastic tube through which water is propelled by an artificial heart, *i.e.*, by a pump of such construction that it discharges its contents into the tube in a manner which mechanically resembles that in which the heart discharges its blood into the arteries. Several instruments of this kind have been contrived, from the simple schema of E. H. Weber, to the complicated "artificial heart" of Marey.

It may be stated generally that those forms of schema are most instructive which are of the simplest construction; and inasmuch as the object in view is not to illustrate but to explain, it is of no importance whatever that the schema should have any outward resemblance to the organs of circulation for which it stands. What is essential in a schema is, that as regards the quantity of liquid discharged at each stroke of the pump, the period occupied in the discharge, the distribution in time of the pressure exercised on the mass of liquid expelled, and the resistance opposed to the terminal outflow of liquid from the elastic tube, the representation should resemble as closely as possible the thing represented. To the student, it is far from an advantage that the resemblance should extend beyond this to the details of external form and arrangement; for his attention is thereby apt to be drawn off from the essential conditions of the *act*, to the accessory peculiarities of the machine which produces it. Two kinds of schema may be usefully employed for the study of the phenomena of the pulse, which differ from each other in the construction of the pump which does the work of the heart. The first is represented in fig. 211. Here the pump consists of a glass tube (A), closed at the upper end, and connected below by two branches—on one side with a cistern, at a level of some eight or ten feet above the table; on the other, with the experimental tube which represents the artery. These communications are controlled by valves, placed at the opposite ends of a horizontal lever (E, D) of such construction that the same act which closes the one must necessarily open the other; so that, as regards their actions, one represents the semilunar, the other the auriculo-ventricular valves of the heart. By means of a spring (shown in the figure to the right of D), when the apparatus is not working, *i.e.*, during the period corresponding to diastole, the former is kept closed, the latter open. Under these circumstances, the water rises in the tube, compressing the column of air which it contains in a proportion which is determined by Marriotte's law. If, as in the present instance, the pressure is about one-third of an atmosphere, the volume of the enclosed air is diminished in the proportion of 2 : 3, and so on. When, by depressing the opposite end of the lever, the aortic valve is opened, and the mitral closed, the compressed air suddenly expands, and forces the water which the tube contains into the aorta. We shall see, when we come to consider the modes of contraction of the heart, that the above is as close an imitation as could be made by any artificial means. Just as, when the heart contracts, it compresses its contents most energetically at the outset, while its force rapidly diminishes towards the end of the systole, so here the most rapid movement of the column is at the first moment after the depression of the lever.

The arterial tube where it passes under the valve D is about four lines in thickness. Soon it divides into two branches of smaller diameter, each of which is several yards long. One of these tubes passes under the spring of the sphygmograph, which is fixed at H in such a manner that tracings may be conveniently taken. Both open finally into a waste basin; but each is provided with screw clamps, by which it can be compressed or constricted at any desired distance from the pump. The purpose of the bifurcation is, that the observer may be enabled, without interfering in any way with the condition of the tube, of which the expansive movements are recorded sphygmographically, to vary the quantity of liquid which is discharged through it per minute. To experiment with the schema satisfactorily, it is desirable to leave the working of the lever to an assistant, or, still better, to arrange the apparatus so that the work can be done by an electro-magnet. The observer is then at liberty to watch the effect of modifications of resistance, &c., on the form of the tracings while they are in progress. The most important facts to be demonstrated with the aid of the schema, as above described, are the following:—

1. It is shown that the artificial and the natural pulse resemble each other closely, each consisting in a succession of expansive and contractile movements which always occur in the same order (*see* fig. 212, *a*). In describing these movements, it is convenient to speak of the experimental tube as the artery, and to assume that *elevation* of the lever of the sphygmograph is equivalent to *expansion* of the tube, and *depression* to *contraction.* This granted, the tracing shows that when the valve D is opened, a sudden expansion of the artery takes place; that so long as the heart continues to act the vessel remains full, and that the cessation of the injection of liquid from behind determines a contraction of the artery which is as rapid as the previous expansion. No sooner has the artery accomplished its contraction than it begins a second expansion inferior to the first both in extent and rapidity; and then finally contracts, continuing to get smaller until the aortic valve again opens.

2. It can next be shown that just as the expansion of the lever is consequent on the opening of the aortic valve, so its descent is consequent (not on the closing of the valve, but) on the cessation of the injection of liquid by the pump, *i.e.*, the cessation of the systolic contraction of the ventricle. To prove this, I use a contrivance which will be readily understood from the figure. Its purpose is to write on the plate of the sphygmograph the duration of the injection of liquid. It consists of a cylinder of box-wood (fig. 211, H), the steel axis of which rests horizontally on bearings so placed that the cylinder revolves in a direction at right angles to that of the movement of the plate at a short distance from it. From one side of the cylinder a steel needle projects, which, when the cylinder turns, makes a mark on the smoked surface. Round one side of the cylinder runs a cord of spun silk, the two ends of which stretch, one from either side of it, to the point of a vertical arm (L); this arm springs from the wooden lever already described, by which the valves are opened and shut. Of the two cords, the upper one is rendered partly elastic by the interposition of a short length of caoutchouc. So long as the aortic valve is closed, the needle remains in contact, but the moment the valve is opened, it is withdrawn, and we obtain, first, an upper horizontal line, broken at regular intervals—which are, of course, limited in time

by the opening and closing of the aortic valve—and, secondly, a pulse-tracing (Fig. 212, *b*), which may be compared with it. This exact correspondence between the length of time the heart is acting and the time which elapses between the beginning of the expansion and the commencement of the contraction, affords evidence that the latter is dependent on the former.

3. Lastly, it can be shown that the second expansion is not, as might be supposed, connected with the closure of the communication between the pump and the elastic tube (the shutting of the aortic valve), but is a consequence of the disturbance of equilibrium produced in the tube itself by the act of distension. To demonstrate this, the second expansion must be studied under various conditions and by various methods; among the best is the following:—A narrow tube, closed at one end, and containing air, is connected by means of a T-piece with the experimental tube or artery. The volume of air contained in the tube varies with the pressure, indicating its variations with great sensitiveness. If the surface of the liquid in the tube is watched during the action of the pump, it is very easy to see that the volume of air is diminished as the valve D opens, enlarges for a moment, and again contracts after the injection has ceased. If now the action of the pump is so modified that, after opening the valve D, the discharge of liquid is continued for some seconds (both valves remaining open), we learn that the first expansion is followed by a second just as before. If the same experiment is made with the sphygmograph, a tracing is obtained in which the ascent due to the opening of the valve is succeeded by a momentary descent, then a second ascent, the lever finally assuming a position corresponding to the increased pressure produced by the continuous current which is now passing through the tube.

From this expcrimnet we learn, as regards the artificial artery, first, that the second beat of the pulse is not, as has been sometimes imagined, a mere product of the instrumental method we employ to demonstrate it, for it can be shown quite as distinctly in other ways; and secondly, that it is a result of the disturbance produced in the tube by the sudden distension of its proximal end, independently of any subsequent movement or action of the pump.

41. **Experiments with the Schema relating to the Form of the Arterial Pulse.**—In the schema, the injection of liquid by the artificial heart into the proximal end of the elastic tube produces two effects, which can not only be distinguished in the tracing, but can be proved experimentally to be independent of each other. One of these consists in the transmission of a series of vibratory movements of the liquid (*i.e.*, movements in alternately opposite directions) from the proximal to the distal end; the other, in the communication of the pressure existing in the artificial heart at the moment

that the valve D is opened to the contents of the arterial tube. The first of these effects can be readily demonstrated on the schema.

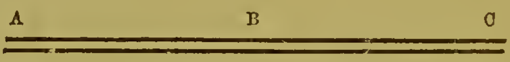
If you take an elastic tube, distended with water, and closed at both ends, and give it a smart rap with a hammer at one end, an effect is transmitted along the tube which, although of an entirely different nature to that which constitutes the pulse, yet mixes itself up with it under certain conditions. This effect is called, from its mode of origin, a percussion-wave. To produce it, close the communication between the schematic heart and artery, and arrange the lever (fig. 211) in such a manner that, by striking on it with a hammer (at D), the required percussion may be produced. The tube being placed under the spring of the sphygmograph (at G), in such a position that the length of tubing between the point of percussion (D) and the spring (G) is equal to two meters, a succession of percussion-waves is produced, and a tracing obtained similar to those shown in fig. 213, in which the interruptions in the upper line indicate the moment of percussion, the vertical ascents in the lower line the effects. In the figure, the interval of time between cause and effect corresponds to the portion of the horizontal line (in the lower tracing) which lies between the short vertical scratch and the commencement of the ascent. The rate of movement of the clock-work during the experiment being 8 centimeters per minute, this distance corresponds to about a fifteenth of a second.

The other effect, the communication of pressure from the artificial heart to the elastic tube, may be readily illustrated with the aid of a schema in which the heart is represented by an elastic bag of such size that it can be squeezed with the hand. This bag communicates at one end with a long elastic tube representing the arterial system, at the other with a vessel containing water, the apertures being furnished with valves which open in directions corresponding to those of the heart. If three levers, like those we have just been using, are so arranged as to receive the successive expansion-waves—produced by repeatedly squeezing the bag—at different distances from their origin, the three tracings are obtained which are represented in fig. 214. It is instructive to observe that these tracings have no resemblance to those of the arterial pulse. The reason is, that the contracting hand is entirely unlike the contracting heart. The real heart, like the schematic heart used in the previous experiments, contracts suddenly, exerting its greatest vigour at the commencement. The hand contracts gradually, and is, moreover, incomparably weaker, as compared with the resistance to be overcome, than the heart. Hence the expansion of the tube is slow, lasts a long time, and is followed by no rebound. This very slowness of the process enables one to see the steps of it better. In the distal part of the tube, to which the upper tracing corresponds, the expansion culminates later than in the proximal part, because the motion communicated to its contents by the grip of the hand at the outset does not begin to tell on the former (distal) until the latter is fully expanded.

In the pulse tracings obtained with the schema arranged as in fig. 211, so as to imitate the natural pulse, the two effects produced in the preceding experiments separately, are combined with each other. Thus in figure 212 *a*, the abrupt initial ascent of the lever is the first of a series of vibratory movements of the same kind as those shown in fig. 213, and is instantly followed by a recoil. In the same tracing, the more gradual accumulation of arterial pressure manifests itself in the fact that the lever jerked up[*] by the vibration does not (as in fig. 213) descend to its previous position, but remains elevated for a period, which, as already seen, depends on the duration of the injection of liquid.

This combination of effects is seen with equal distinctness in the natural radial pulse. The abrupt line of ascent with which every normal tracing begins, expresses not the more or less gradually increasing arterial distension, but the antecedent transmission of a vibration.

42. Postponement of the Pulse.—There is a sensible difference in time between the beat of the carotid artery and that of the radial. Any one can satisfy himself of the fact by feeling his own carotid with the left thumb and fore-finger, while he feels the left radial with the other hand. The reason why time is lost in the transmission of the expansion from the centre to the periphery, is that the arteries are elastic. Let us suppose a tube, A, B, C, to represent the arterial system—A the proximal end, C the distal. At the instant that blood bursts suddenly out of the contracting heart into A, it yields to the pressure against its internal surface and expands. In this expansion great part of the sensible motion of the blood momentarily disappears, and consequently, so long as the expansion lasts, produces comparatively very little effect in distending B; but immediately that A becomes tense, the lost, or rather converted, motion again becomes sensible, and adds itself to the motion which the contracting heart is still communicating. And, inasmuch as B deals with the accumulated effect which it receives from A in exactly the same way as A dealt with that which



[*] In the sphygmographs, lately made by Bregnet, the movement of the spring is communicated to the writing lever by a mechanism shown in fig. 209 *b*, more simple and effectual than that described on p. 218. The screw is hinged to the upper surface of the spring in such a way that it presses gently against the axis of the lever, and acts upon it as a rack on its pinion. In this way the lever follows the movements of the screw much more exactly, and the jerk is diminished. (*See* Garrod on Sphygmography. Journ. of Anat. and Phys., May, 1872, p. 399).

it received from the heart, C is as far behind B in attaining its maximum of distension as B was behind A. This being the case, it is easy to see that the loss of time between A and C, or between aorta and radial, depends on the yieldingness (extensibility) of the tube by which the two points are connected. If the tube is absolutely rigid, there is no postponement; if, though elastic, it is tense at the moment that it receives the discharge, there is scarcely any; whereas that condition of the tube is most favourable to postponement, in which it is longest in attaining its maximum of distension, or in which the time taken by any part of it to expand to the uttermost is longest.

The preceding explanation relates exclusively to so much of the pulsation as is due to the communication of pressure. As regards the antecedent vibration-effect, we have also time occupied in transmission, but the rate of propagation is so rapid that in the case of an artery (or of an elastic tube of similar length) it is inappreciable. This fact enables us to explain how it is that in some persons the pulse seems to be much more postponed than in others. The reason of this is, not that there is more time lost in the former case than in the latter (for even if this were so, the difference would be certainly too inconsiderable to be judged of by the finger), but that in some individuals and under certain conditions of health, the instantaneously transmitted vibration-effect is more felt by the finger; in others, the moment at which the artery attains its greatest extension. Thus a pulse of the form shown in fig. 215 *a*, seems to the finger *delayed*, because the vibration-effect is in abeyance on account of the existence of an obstruction between the heart and the wrist; whereas in the pulse represented in *b*, the initial shock is so intense that it masks the other.

43. **Cause of the Second Beat.**—The facts relating to the postponement of arterial expansion are also the key to the understanding of the phenomenon of dicrotism. In applying them in explanation of the production of the second expansion in arteries which, like the radial, are not far from the periphery, there are two facts to be borne in mind; first, that these arteries, as they become smaller, become more distensible; and secondly, that in the capillaries themselves the resistance to the passage of blood is much greater than any which is encountered in the arteries. Just as the expansion of the aorta determines that of the radial, the radial expansion determines and is followed by that of the peripheral arterioles. Hence at a certain moment the radial is subsiding, while the arterioles are still swelling; so that when they are at their acme of distension, the pressure is greater at the periphery than in the radial itself. From the other fact—the resistance to the flow of blood in the capillaries—it results that, immediately behind this resistance, pressure accumulates so long as blood enters the arterioles from behind more rapidly than it

is discharged in front. The state of the arterial circulation during the period of cardiac diastole, may therefore be described as follows:—The arterial system is closed by the aortic valve behind, and virtually closed in front by the capillary resistance. In the largest arteries the expansion is ebbing, in the smallest it is culminating; so that, for an instant, the pressure is greater in the latter than in the former. There is but one effect possible. The restoration of equilibrium must take place by increase of pressure towards the heart and diminution towards the periphery. This restoration of equilibrium constitutes the second beat. It may manifest itself in very different degrees, according to the yieldingness of the arteries. When, as in health, the arteries are tense, it is seen merely in a slight arrest or interruption of the arterial collapse—a break in the descending limb of the tracing. In fever, when the arteries are relatively much more distensible, the second expansion is separated by so distinct an interval of relaxation from the first, that the pulse feels double to the finger. To facilitate the comprehension of the subject, the synchronous conditions of central, peripheral, and intermediate arteries may be stated in parallel columns.

Carotid.	Radial.	Peripheral arterioles.
Fully expanded .	Expanding . .	Collapsed
Contracting . .	Expanded . .	Expanding
Again expanding .	Contracting . .	Expanding
Stationary . .	Again expanding .	Slowly contracting
Contracting . .	Contracting . .	Contracting

Hence, as sphygmographic tracings show to be the case, the second expansion in the great arteries lasts longer than in the smaller ones; for, although it commences the sooner, the nearer the heart, the subsidence is simultaneous throughout the whole arterial system.

Rules for Sphygmographic Observation.—1. The fore-arm should be supported on a table or other similar surface, with the back of the wrist reposing on a firm, well-padded cushion, of such a height that the dorsal surface of the hand makes an angle of from 20° to 30° with that of the fore-arm.

2. The sphygmograph must be placed on the wrist in a direction parallel with that of the radius, in such a position that the block rests upon the trapezium and scaphoid, and the extremity of the spring is opposite the styloid process of the radius.

3. In beginning an observation, adjust the instrument so that the pressure exerted by the spring is sufficient to flatten the artery against the radius; then weaken the spring until the effects of over-compression disappear—*i.e.*, until you find that the lever continues to descend until the end of diastole. Note the pressure at which this result is attained, as well as that which is required to flatten the artery, and take tracings at each of the two pressures.

Section III.—Phenomena of the Circulation in the smallest Arteries.

The smallest arteries may be studied during life with the aid of e microscope, in fish, batrachians and mammalia.

44. For the microscopical study of the circulation in fish, a contrivance devised by Dr. Caton, of Liverpool, is used (fig. 216). It consists of an oblong box of gutta percha, open at one end, closed at the other, and just large enough to hold the body of a minnow or stickleback very loosely. This box forms part of a plate of gutta percha, which is fixed on to the stage of the microscope in such a position that the tail of the fish contained in it covers a perforation in the plate prepared for its reception. The tail is held securely in its place by a ligature, and the caudal fin which rests on a square of glass is further secured by a couple of fine springs. The box itself, which encloses the head and gills of the fish, contains water, which is constantly renewed by means of the two tubes, of which the upper, guarded by a screw-clamp, communicates with a vessel at a higher level, the lower conveys the water away as fast as it is supplied. The excellency of this method lies in the fact that the animal can be kept under observation, without the use of any narcotising drug, for a long time in a perfectly natural condition. The frog is used both in the larval and adult state. To observe the circulation in the tail of the tadpole, the animal is placed in a moderately strong solution of curare, care being taken to remove it before it is completely paralysed—the moment, in short, that its motions become sluggish. It is also possible to secure it, without the aid of curare, in a holder of construction similar to that of the instrument I have just described—a method which has this great advantage, that the animal is in a more normal condition; for even when curare is given with the greatest care, the action of the heart is weakened by it. For most purposes the adult frog is more useful than the tadpole, particularly when it is desired to observe not merely the circulation as it is, but to witness the modifications which the phenomena undergo under the influence of conditions acting on the blood-vessels through the nervous system.

There are three transparent parts of the frog—the mesentery, the web, and the tongue—each of which has its special advantages for the purposes of study. For a first view of the relation between arteries, capillaries, veins, and lymphatics, the mesentery is superior to either of the other two. The frog must be placed under the influence of curare, the dose of which, for the ordinary specimens of *rana temporaria*, is about $\frac{1}{2000}$th of a grain. The solution of curare is prepared by weighing out five milligrammes of the substance, and rubbing it up in a glass mortar with a little alcohol. The proper quantity of water—that is, sufficient to make up ten cubic centimeters—is then added, and a straw-coloured, nearly limpid liquid is obtained, a single drop of which is a sufficient dose. It is injected under the skin of the back with an ordinary subcutaneous syringe, and answers best when the effect does not manifest itself for some time after the injection. The most convenient apparatus for the purpose of exposing the mesentery is that shown in fig. 217. The manipulation is fully described in Chapter VII. p. 168. It is always desirable to commence the examination with a low power. It is then seen that the arteries are smaller than the veins, the latter exceeding the former in diameter by about a sixth; that the arterial stream is quicker than the venous; that it is accelerated appreciably at each beat of the heart; and that in every artery a space can be distinguished within the outline of the vessel, which is entirely free from corpuscles. The arterial stream, indeed, is so quick that the forms of the corpuscles cannot be discerned, but in the veins both coloured and colourless corpuscles can be distinguished; and it is soon noticeable that, while the former are confined to the axial current, the latter show a tendency to loiter along the inner surface of the vessel, like round pebbles in a shallow but rapid stream. The observation may be continued without material change for many hours; but if any artery is measured from time to time micrometrically, it will be found that after a while it becomes wider. On this dilatation of the arteries follows a corresponding though less marked enlargement of the veins, and, if the attention of the observer is fixed upon these last, it is seen that the circulation, which was before so active, undergoes a marked and almost sudden slowing. This slowing indicates that the membrane, in consequence of its exposure to the air, is becoming inflamed; simultaneously with it, the colourless corpuscles, instead of loitering here and there at the edge of the axial current, crowd in numbers against the venous walls. In this way the vessel becomes lined with a continuous pavement of these bodies, which remain almost motionless, notwithstanding that the axial current still sweeps by them, though with abated velocity. If at this moment the attention is directed to the outer contour of the vessel, it is seen that minute, colourless, button-shaped elevations spring from it, each of which first assumes the form of a hemispherical projection, and is eventually converted into a pear-shaped body, attached by a stalk to the outer surface of the vein. This body, which has thus made its way through the vascular membrane, is, I need scarcely say, an amœboid colourless corpuscle. It soon shows itself to be so by throwing out delicate prongs of transparent protoplasm from its surface, especially in the direction from which it has come.

The methods to be employed for the study of the circulation in the tongue and in the web are fully described in Chapter VII. For investigations relating to the innervation and contractile movements of the smallest arteries, the tongue is of little value, though superior to the mesentery and web for the study of inflammation. The web, on the other hand, is preferable, for the purposes first mentioned, to either the tongue or mesentery.

45. **Capillary Circulation in Mammalia.**—The study of the capillary circulation of mammalia under the microscope is attended with great difficulty—in the first place, because (if we except the wing of the bat) there is no external part sufficiently transparent for observation under high power; and, secondly, because if internal parts are used, the injurious effects of exposure are much greater than those which occur in batrachians. To overcome these difficulties, it is necessary to have recourse to more complicated appliances and apparatus.

The mesenteries of small rodents have been repeatedly used for the demonstration of the mammalian capillary circulation. These, however, are not to be compared, as subjects of observation, with the omentum, and particularly with that of the guinea-pig. This structure forms a delicate membranous expansion of from twelve to fifteen cubic centimeters in extent, which is attached by its upper margin to the greater curvature of the stomach. It differs from the organ of the same name in man in consisting, for the most part, of only two layers of peritonæum, in being much more delicate in its structure, and containing very little fat. Hence, from the simplicity of the anatomical relations, and particularly from its being attached by one side only to the stomach, from its perfect transparency, from its abundant vascularity, and, lastly, from its containing not only vessels but living cells, it is obvious that this membrane offers a good field for research.

The observations hitherto made on the mammalian mesentery have been without practical result, the reason being that so vulnerable a tissue as that of the peritonæum cannot be exposed, even for a few minutes, without injury; so that, although the greatest care is taken in demonstration, only a momentary glimpse can be obtained. To obviate this difficulty, the arrangements for placing the membrane under the microscope must be of such a nature, that the structure is bathed during the whole period of observation in a liquid at the temperature of the body. It need scarcely be said that water, from its destructive influence on living tissues, would not answer the purpose. Serum would probably be best, if it were always at hand; but, practically, solution of common salt of the strength ordinarily used ($\frac{3}{4}$ per cent.) answers the purpose perfectly. The temperature is maintained by keeping the glass trough, in which the membrane is spread out, over the warm stage, the construction of which has been already described.

The mode of procedure is as follows:—The guinea-pig is first placed under the influence of chloral by injecting that substance in solution under the skin, three grains being required for an animal about 1lb. in weight. It is then laid on a support, the upper surface of which is on the same horizontal plane as that of the microscope-stage. An incision not more than an inch in length is next made, extending outwards from the edge of the left rectus muscle a little below the end of the ensiform cartilage. The muscles having been divided, and the peritonæum cautiously opened for about half an inch, or even less, the free edge of the omentum is carefully drawn out. It must then be floated in the warm bath prepared for it, and is ready for examination. It is, however, found very advantageous to cover those parts of it which do not lie under the microscope with sheets of blotting-paper, for by this means the risk of exposure is diminished, and the undulating movements of the water are prevented; so that the object is rendered much steadier than it would otherwise be. So long as low powers are employed, this arrangement is sufficient; but if it is desired to use objectives of short focal distance, it is necessary to warm the objective by allowing a stream of water from the same source as that which supplies the stage to pass round it.

The objects which present themselves to the observer are manifold. Veins and arteries may be studied of various diameters, some of which are free, while others are surrounded by sheaths of tissue in which there are labyrinths of capillaries of surpassing beauty. Several new observations have already been made by this method. One of the most important, physiologically, is the fact that the maintenance of the capillary circulation is wonderfully dependent on temperature; and, in particular, that any rise of temperature above the normal is in the highest degree injurious, partly, perhaps, from its direct influence on the blood corpuscles, but mainly because it produces changes similar to those we have already noticed as occurring in batrachians after long exposure—viz., arrest of the capillary blood-stream and escape of the liquor sanguinis and corpuscles into the surrounding tissue.

46. **Artificial Circulation.**—For many purposes of research, it is desirable to observe the circulation independently of the action of the heart. This is accomplished either in the whole body or in an organ, by injecting blood, or a liquid which may be substituted for it, in a constant stream into the arterial system, at the same temperature and under the same pressure as that which naturally exists in the arteries. In the case of batrachians, this is accomplished without difficulty, for the temperature of the body differs little from that of the atmosphere, and the nutritive processes can be maintained for long periods, not only without respiration, but without the agent by which oxygen is conveyed to the tissues—

hæmoglobin. Consequently the conditions to be observed are very simple. The requirements for the purpose are as follows:—

1. The liquid to be injected may be either serum, defibrinated blood, or ¾ per cent. solution of chloride of sodium. When serum is used, it must be absolutely fresh. For this reason, the serum obtained from the slaughter-house is usually not to be depended upon. It is therefore necessary to use a small rabbit for the purpose. In order to obtain a sufficient quantity of blood from this animal, a cannula must be carefully secured in the carotid, and a clip placed on the artery. The connector adapted to the cannula must be of sufficient length to reach an absolutely clean flask or capsule destined for the reception of the blood. If serum is required, the capsule must be allowed to stand in a cool place until it is coagulated. If defibrinated blood, the flask must be agitated briskly immediately after it is collected. The blood should be taken in successive portions, for in this way a much larger quantity is obtained than would be yielded if the animal were allowed to bleed to death at once.

2. The apparatus for injection consists of a funnel, supported on a holder at a height of about two feet from the table, to the stem of which a flexible tube, guarded by a clip, is adapted. In addition to this, two cannulæ must be prepared, one for the *bulbus arteriosus,* the other for the *vena cava inferior.* Both should be made of thin fusible glass, and of the size and form shown in figure 218. The arterial cannula must be connected by an india-rubber tube of the same width as itself with a glass joiner, and its end must be supported by a holder, which can be best made of a strip of sheet lead bent to the proper form. The funnel having been filled with the liquid to be injected, and connected with the cannula by the joiner, a sufficient quantity is allowed to flow into the tube to occupy it completely, and the clip closed. All being now ready, a frog, previously slightly curarized, is fixed on the table in the supine position. The integument is divided over the sternum in the middle line, and the anterior wall of the upper part of the visceral cavity removed, so as to expose the pericardium, great care being taken not to injure the abdominal vein, or any other large vessel. The ventricle is then opened, and the cannula passed through the opening into the bulb, and secured by a ligature. This done, the heart is drawn upwards, and to the right (after severance of the small vein which stretches from the back of the ventricle to the pericardium), so as to expose the *sinus venosus,* which is then opened in the line of junction between it and the auricles. By this opening, the cannula for the vena cava is easily introduced into the funnel-shaped dilatation (*see* fig. 228 *b*), and pushed into the vein. If the cannula is of proper size, a ligature is unnecessary. On opening the clip on the tube

leading from the funnel, the circulation is restored. The blood contained in the vascular system of the animal is soon replaced by the liquid injected.

The most instructive observations, relating to frogs in which the circulation is maintained artificially (sometimes called salt or serum frogs, according to the liquid used), are made with the aid of the microscope. The examination of the web shows us that even when saline solution is used, the vessels and the circulation through them remain unaltered for some time. If serum is used, this period is longer, provided that it is perfectly fresh. A very slight admixture, however, of kept serum is fatal to the experiment. After a time, decline of tissue life manifests itself by a change in the appearance of the preparation, the elements losing their plumpness and distinctness of outline. Along with this change, the vessels, and particularly the arteries, become relaxed, and the normal exchange between the liquid inside and that outside of the vessels is perverted, the latter increasing in such a way as to render the whole animal œdematous.

If, while the circulation is still normal, an injury is inflicted on a part of the web—as, for example, by applying mustard to a spot on its surface—it is seen that in the injured part changes occur suddenly which are analogous to those which, as tissue death approaches, affect the whole body. These changes are known by the term *stasis*, and form part of the process of *inflammation*—a word which is used as a general expression for the local effects of injuring living parts to such a degree as not to destroy their vitality at once. They are best studied when serum which contains a few corpuscles, or defibrinated blood diluted with saline solution, is employed. It is then seen that in any part of the web to which a so-called irritant is applied, as, *e.g.*, mustard—the blood stream is retarded, and the corpuscles crowd together in the dilated vessels. This is not due to any property of mutual attraction peculiar to the corpuscles, for the same thing happens if milk, diluted with saline solution, is substituted for blood; so that whatever be the nature of the change, its seat is not in the circulating liquid itself, but in the vessels or surrounding tissues.

SECTION IV.—FUNCTIONS OF VASOMOTOR NERVES.

In the preceding section the arteries have been regarded merely as passive elastic tubes, dilating or contracting according to the pressure exercised upon them by the circulating blood. They must now be studied as not only elastic but contractile.

The arteries owe their contractility to the unstriped muscular fibres which they contain. These fibres shorten under the influence of impressions conveyed to them by the vascular nerves, which nerves, together with the automatic centre from which they radiate, constitute the vasomotor nervous system. Of the centre which governs arterial contraction, nothing is known anatomically; for there is no point or tract in the brain or spinal cord to which vascular nerves can be traced back. All that is known has been learnt exclusively by experiment.

That there is a vasomotor centre, and that it is intracranial, we learn by observing, first, that if the medulla is divided immediately below the cerebellum, all the arteries are relaxed, and that a similar effect is produced if certain afferent nerve fibres, which lead to the intracranial part of the cord, are excited. Its position has been lately determined with great precision in the rabbit by Ludwig and Owsjannikow, who have found by experiments, to which further reference will be made, that it is limited towards the spinal cord by a line four or five millimeters above the *calamus scriptorius*, and extends towards the brain to within a millimeter of the *corpora quadrigemina*.

That the vasomotor centre is in constant automatic action, is shown by the paralysing effect of section, whether of the spinal cord, or of any nerve known to contain vascular fibres. If the action of the centre were not constant, division could not produce arterial relaxation. In relation to this constancy of action, the word *tonus* is used. Arterial tonus means that degree of contraction of an artery which is constant and normal. It is maintained only so long as the artery is in communication with the vaso-motor centre.

47. Experiments relating to the Influence of the Cerebro-spinal Nervous Centres of the Vascular System.—(1.) Destruction of the Nervous Centres.— Two frogs are slightly curarized, and placed side by side on the same board, in the supine position. In both, the heart and great vessels are exposed, as in the preceding section. It having been ascertained that the circulation is normal in each animal, and the frequency of the contractions having been noted, the brain and spinal cord are destroyed in one of the frogs, by inserting a strong needle into the spinal canal immediately below the occipital bone, and then passing it upwards and downwards. This may usually be accomplished without much loss of blood. If now the frog which has been deprived of its nervous centres is compared with the other, it is seen that in the former, although the heart is beating with perfect regularity and unaltered frequency, it is empty, and in consequence, instead of projecting from the opening in the anterior wall of the chest, it is withdrawn upwards and backwards towards the œsophagus.

The emptiness of the heart is not limited to the ventricle and bulb. The auricles are alike deprived of blood; and if the heart is drawn forwards by the apex, it is seen that the *sinus venosus* and *vena cava inferior* are in the same condition. The state of the heart is therefore not dependent on any cause inherent in itself, but on the fact that no blood is conveyed to it by the veins. To make this still more evident, the rest of the visceral cavity may be opened, when it is seen that, although the vena cava is collapsed, the intestinal veins are distended. The second frog, which is no longer required for comparison, should now be pithed in the same manner as the first. A cannula is then introduced into the abdominal vein, with its orifice towards the heart, and connected, by an india-rubber tube guarded by a clip, with a funnel containing $\frac{3}{4}$ per cent. solution of chloride of sodium. The heart having been exposed, and its empty condition noted, the clip is opened. Its cavities at once distend, and it acts as vigorously and effectually as before the destruction of the nervous centres. The experiment may be varied thus:—Two frogs are suspended side by side, one of which has been pithed in the manner above described. In both, the heart is exposed and the ventricle cut across. In the pithed frog, a small quantity of blood escapes, the quantity contained in the heart itself and the commencement of the arterial system. In the other, blood continues to flow for some minutes in consequence of the continued contraction of the arterial system. To what extent the veins may participate in it is uncertain.

These simple experiments show, first, that in the frog the arteries, unaided by the heart, continue the circulation for a certain time after equilibrium of pressure has been established, by virtue of their contractility; and secondly, that in this animal the influence of arterial contractility in aid of the circulation is so considerable, that when it is abolished, circulation is no longer possible.

It may be well to point out that this fact affords no ground for supposing that the arteries take any active part in maintaining the circulation. All that is proved is, that in the relaxed state the vascular system of the frog is relatively so capacious, that it is more than large enough to contain the whole mass of the blood, which consequently comes to rest in it out of reach of the influence of the heart. During life, the arterial tonus is usually constant; so long as and in so far as this is the case, the function of the arteries is a passive one, the motion they give to the blood-stream during diastole being a mere restitution of that received by them from the heart during systole. On the other hand, whenever they contract, they originate motion of themselves; but in this case the duration of the effect is limited by that of the contraction, and can never be continuous.

48. (2.) Direct Excitation of the Spinal Cord in the Frog.—The requirements are as follows:—*a.* A thin board of soft wood, about 8 inches long and 2 inches broad, one end of which has a V-shaped notch cut out of it, corresponding in form and size to one of the interdigital membranes of the web of the frog's foot. *b.* A pair of common strong sewing-needles; around the blunt end of each of these needles, the end of a length of thin copper wire is closely coiled; they are then covered nearly to their points with a protective and insulating coating of soft sealing-wax, for which purpose it is necessary to warm them in the flame of a lamp. In doing this, care must be taken not to heat the point. *c.* A battery and Du Bois' induction apparatus and key. The key must be interposed in the secondary circuit.

A frog having been curarized just sufficiently to paralyse its voluntary muscles, a straight line is drawn from the notch along the upper surface of the board in a direction parallel to its edges. Two small perforations are made in this line, a couple of millimeters from each other, at a distance from the notch equal to that from the web of the frog to its occiput. Through these perforations the needles are thrust, so as to project about 5 millimeters, after which the board is arranged in such a way on the microscope, that the V-shaped notch rests over the stage aperture, and the opposite end on a support at the same level. All being now ready, the integument is opened along the middle line of the back of the neck, and the occipital bone perforated in the middle line with a fine awl, close to its posterior margin. The frog is then laid, back downwards, on the board, in such a position that one of the needles enters the cranium through the hole in the occipital bone, the other the spinal canal. The web is then laid on a plate of glass which covers the notch, and secured if necessary by fine pins. Finally, the heart is exposed as before.

On opening the key for a moment, so as to allow the induced current to pass through the needles, it is seen that all the arteries of the web at once contract, the contraction increasing for four or five seconds and then gradually subsiding. If the excitation is continued for several seconds, the circulation stops. To judge of the effect accurately, it is desirable, first, to fix upon an artery for observation beforehand, and bring it well into view; and secondly, to measure its diameter before, during, and after excitation. For this purpose, a sheet of paper is placed on a board in such a position that its surface is at right angles to the direction in which the image is thrown by the prism (*see* fig. 219), and at a distance of about 10 inches from it. The outlines of the vessel are then traced on the paper with a fine hard pencil. During and after excitation, other tracings are made in the same way; by comparison of which the changes of the diameter of the vessel can be accurately estimated. The microscope must of course be so placed that light is received from the side, and that the surface of the paper is sufficiently illuminated to enable the observer to distinguish the point of the pencil. To ensure success in this fundamental experiment, the following precautions must be attended to. The dose of curare must be very small, and should therefore be given an hour or two before the observation is made. One at least of the electrodes must be inserted within the cranium; for if both are below the occipital bone, the effect is uncertain. Lastly, great care must be taken to use feeble currents, and not to prolong the excitations, for the vasomotor nervous system of the frog is very readily exhausted.

49. (3.) **Excitation and Section of the Spinal Cord in the Rabbit.**—The requirements and preliminary preparation for this experiment are the following:—A cannula and subcutaneous syringe for injecting 20 per cent. solution of curare into the jugular vein; apparatus for a kymographic observation of arterial pressure; apparatus for artificial respiration; a needle for ligaturing the muscles, in addition to the ordinary instruments. The cannula for the jugular is shewn in fig. 220. An india-rubber tube is fitted to it, the end of which is closed by a ligature. It is inserted as follows:—The rabbit having been placed in the usual way on Czermak's rabbit supporter, with the cushion under its neck, the integument is divided in the middle line from the *pomum Adami* downwards, as directed in Section I. On drawing the edge of the incision to either side, the external jugular vein is readily seen as it crosses the sterno-mastoid. It is then carefully cleared of the platysma fibres and fascia which cover it, and of its sheath to the extent of an inch or more, with the aid of two pairs of blunt forceps. A clip having been placed on the proximal end of the cleared part, a ligature is looped round the distal end, which is tightened as soon as it is seen that the vein is distended. This being accomplished, a second ligature is placed round the vessel between the first ligature and the clip, and then a V-shaped incision is made in the vein immediately beyond it. Finally, the cannula, which has been previously filled with saline solution, is slipped into the vein and secured in its place by the ligature prepared for it. When it is intended to inject, the point of the subcutaneous syringe is thrust through the closed tube of india-rubber. On withdrawing it, no liquid escapes. The plan has the advantage that successive quantities may be injected with the greatest facility. The mode of preparing the carotid artery, and of connecting it with the kymographic cannula, has been described in § 34. For the present purpose it is necessary to free the artery from its connections to a greater extent than usual. The cannula having been secured in the artery, and the latter divided beyond the point of

insertion, the cannula is turned back and fixed to the animal's thorax (by tying it to the fur) in such a position that the artery forms a loop, with its convexity towards the head. The purpose of this arrangement is to prevent the artery from being strained when the animal is turned.—The apparatus for artificial respiration has not yet been described. It is required because the animal being under the influence of curare, its voluntary muscles are paralysed. As a substitute for natural breathing, air must be injected in the proper quantity at regular intervals, which correspond with the previous frequency of the respiratory acts. In the absence of self-acting apparatus, the best instrument to use is the caoutchouc blower and expanding regulator sold by Messrs. Griffin for working the gas blow-pipe (*see* fig. 221). The blower is worked by means of a squeezer. It consists of an oblong board or lever, 16 inches long, 3 inches wide, and ¾ inch thick. This board is hinged in the middle to a fulcrum, in such a way as to admit of a see-saw movement. The fulcrum is firmly screwed to the table. When it is in use, the blower is placed under one end, *i.e.*, between it and the table, the degree of compression being limited by a strong cord attached at the opposite end to the table. By varying the length of the cord, the quantity of air injected at each stroke is regulated. The blower communicates with the respiratory cavity by a tracheal cannula. No valve is required, the expired air passing out freely during the intervals between each injection and its successor, by a hole in the tube. The quantity of air discharged by the blower at each stroke must, therefore, considerably exceed the quantity which is required for respiration. This contrivance can be worked with much less fatigue than bellows. The time must be regulated by a metronome.—The *self-acting apparatus* consists of two parts—a constantly acting blower or expirator, and an arrangement for interrupting the current of air at regular intervals. The best constant blower is that known as Sprengel's blow-pipe,* the structure of which will be understood at once from fig. 222. The essential part of it is the vertical tube *d*, with its branch *e*, the lower end of which opens into a bottle having two other openings. Of these, one, which communicates with the top of the bottle, is for the efflux of air; the other, near the bottom, for the escape of water. If a continuous current of water is caused to pass through *d*, *e* remaining open, it carries with it a quantity of air which passes down into the bottle; and if the screw clamp *c* is so adjusted as to allow the water to flow out of the bottle at the same rate that it flows in from *a*, the water in the bottle remains at the same level, and a constant stream of air escapes from *b*. The interruption of the stream of air so produced is effected by means of an electro-magnet, which is so arranged that each time the voltaic current is closed, a weight by which the tube is compressed is lifted, and thus air is injected so long as the magnet is in action. The voltaic current may be closed and opened either by a metronome or by the mercurial breaker, shewn in fig. 223. Two copper wires, one of which is connected with the battery, the other with the magnet, run along the top of the wooden bridge, nearly meeting at the crown of the arch; here they descend parallel to each other, but not in contact. Below the arch is a flat vulcanite bag, on the upper surface of which a U tube is supported vertically, with its concavity upwards. The ends of the two wires are received into the two limbs of the U. As the bend contains mercury, it is obvious that whenever the bag expands the circuit is closed, and broken when it contracts. The rest of the mechanism is so arranged that the tube is closed beyond the breaker whenever the magnet is not acting, and open so long as the current passes. This condition can, however, never be permanent; for after an interval of time, which can be very readily regulated by altering the quantity of mercury in the U tube, the bag becomes sufficiently distended to close the circuit. When this happens, the magnet acts and opens the tube, allowing the distended bag to discharge itself. This contrivance answers particularly well for the artificial respiration of rabbits. The needles for exciting the cord are constructed in the same manner as those described in the preceding paragraph; they should, however, be thicker and stronger.

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* A somewhat more complicated apparatus (*Wasserluftpumpe zur Erzeugung comprimirter Luft*) is sold by Desaga of Heidelberg.

The cannulæ having been placed in the trachea and external jugular vein, and the apparatus for artificial respiration being in order, three-tenths of a centimeter of a one per cent. solution of curare is injected. As soon as respiration ceases, air is injected at regular intervals by the metronome, the beats of which express the previous frequency of breathing. The carotid artery is now connected with the kymograph, and the animal placed in the supine position, the head-holder being so arranged that the head is very much flexed on the cervical part of the spinal column, so as to make the space between the occipital bone and the atlas as wide as possible. In doing this, great care must be taken not to strain or twist the artery, or kink the air tube. This done, an observation must be made of the arterial pressure, and the atlanto-occipital membrane exposed with as much despatch and as little bleeding as practicable. This is best effected with the aid of the notched needle, fig. 203 *f*. With the help of this needle, three ligatures are passed underneath the muscles which stretch vertically on either side of the spine of the atlas, its point being directed towards the occipital spine as close to the bone as possible. It is usually necessary to pass two such ligatures in line on either side, the upper entering where the lower passes out. The ligatures having been tightened and the muscles divided in the middle line, it is easy to expose the posterior tubercle of the atlas, the membrane, and the edge of the occipital bone, without hæmorrhage.

The next step is to expose the cord by dividing the atlanto-occipital membrane; this is best done with scissors and forceps. While a tracing of the arterial pressure is taken by an assistant, the cord is divided: at once the mercurial column sinks from, say, 100 millimeters to 20 or 30. One needle is then inserted in the middle line above the posterior tubercle of the atlas, the other below it, the key being closed. On opening the latter so as to direct the induced current through the needles, the arterial pressure rises to a height which at first equals, if not exceeds, that at which it stood before section.

The effects of exciting the cord in increasing the arterial pressure are seen with equal distinctness when the cord is not previously divided. In both cases the ascent is accompanied with an increase of the frequency of the contractions of the heart, the cause of which will be investigated in a future section.

Direct Observation of the Arteries during Excitation of the Cord.—That the increase and diminution of arterial pressure observed is in great part, if not entirely, dependent on contraction of the arterial systems, can be shown in several ways. The most direct consists in the observation of the arteries themselves. In the rabbit, the *arteria saphena*, which, after leaving the femoral, just as that vessel enters the adductor sheath, takes a superficial course towards the inner side of the knee, may be observed with great facility. All that is necessary is to divide carefully, first the skin, and then the fascia which covers it: the two saphena veins which lie on either side of it serve to determine its exact position. In this artery it can be readily seen that as the pressure rises the vessel contracts. To observe the effect of vascular contraction on the heart, that organ must be exposed. In a curarized animal, this can be effected without interfering materially with the vital functions. Ligatures of fine copper wire having been passed, with the aid of a curved needle (fig. 203, *e*), around the 3rd, 4th, 5th, and 6th cartilages, close to the left edge of the sternum, and a second vertical series of ligatures around the corresponding ribs at a sufficient distance outwards, the portion of the thoracic wall which lies between the two series can be removed without hæmorrhage. It is then seen that after section of the cord, the heart is flaccid and empty, and that its cavities fill and its action becomes vigorous when the vascular contraction caused by excitation of the peripheral end forces the blood forwards so as to fill the right auricle.

[For the experimental proof that the effects of excitation of the cord above described are not dependent on the increased vigour of the contractions of the heart, *see* § § 80, 81.]

50. (4.) **Section of the Medulla Oblongata in the Rabbit, within the Cranium.**—The recent experiments of Ludwig and Owsjannikow have shewn that the medulla may be divided within the cranium with the same results as regards arterial pressure as are obtained when it is severed immediately below the occipital foramen. For this purpose, the occipital bone must be perforated with a small trephine (fig. 203, *d*) in the middle line between the occipital protuberance and the occipital spine (*see* fig. 224). By this opening, a thin-bladed knife is introduced in the middle plane, with its edge outwards, by which the medulla is divided, first on one side, then on the other. If the division is made as much as five millimeters above the calamus scriptorius, the diminution of arterial pressure produced is as great as after section outside of the cranium. In experiments in which the division was made higher, the effect was found to be lessened, disappearing when a point was reached about a millimeter below the corpora quadrigemina.

Experiments relating to the Reflex Excitation of the Vasomotor Centre.

The vasomotor centre, although constantly in activity, may be stimulated by impressions received by it through afferent nerves. This can be shown both in the frog and in mammalia.

51. **Reflex Excitation of the Medulla Oblongata in the Frog.**—For this purpose, the nerves in question may be excited either with the aid of the ordinary excitor (fig. 225), or by the application of a metallic brush to the skin. In the latter case, one of the wires which form the secondary circuit ends in a point which is inserted into the muscles; the other, in the brush which is kept in contact with the skin in the immediate neighbourhood. The effect should be observed in the web, in the mesentery, and in the great vessels leading to the heart. The currents employed must be feeble when the nerves are excited by the direct application of the electrodes to the sensory nerves, but strong when it is intended to excite their cutaneous or mucous endings. The periods of excitation should always be very short. The experiment may be varied as follows:—*a.* A frog having been carefully curarized, with the same precautions as were recommended for studying the effect of direct excitation of the medulla, and arranged for the microscopical observation of the circulation in the web, the points of the excitor are placed upon the tongue, the mouth being kept open for the purpose. On opening the key, the same changes exactly are observed in the vessels as are produced by direct excitation. At the first moment the blood-stream in the arteries is accelerated, but immediately after, the arteries begin to contract sensibly. The contraction increases gradually but rapidly for one or two seconds, and is attended with slowing, and finally with arrest, of the circu-

lation. A maximum of narrowing having been attained, the effect passes off as it came on. Even if the excitation is continued, the arteries do not remain contracted, but often exhibit alternations of contraction and relaxation at irregular intervals. For observing the changes of rate of movement in the velocity of the blood-stream, the veins should be preferred; for in them the initial acceleration is not quite so transitory as in the arteries, while the subsequent slowing is as distinct. If it is desired to make a more exact observation, the method devised by Dr. Riegel must be used. It consists in comparing the movements of the blood corpuscles in a selected artery or vein, with that of a current of water containing solid particles in suspension, which passes through a horizontal glass tube fixed in the eye-piece of the microscope at such a distance from the eye-glass as to be distinctly seen by the observer. One end of the tube communicates with a large bottle placed on a shelf at a higher level than the table, containing the liquid; the other, with the discharge tube of the movable warm stage represented in fig. 3. By varying the height of the dropper, the rate of flow through the eye-piece can be readily regulated. The rate of flow is learnt by measuring the quantity of liquid discharged per second, and dividing it by the product of the lumen of the glass tube and the magnifying power of the microscope. Thus, if the rate of discharge were a cubic centimeter in 15 seconds, *i.e.*, 6·6′ cubic millimeters per second, the lumen of the tube 0·8 square mill., and the magnifying power 300, the velocity of the current would be $\frac{6·6'}{300 \times 0·8}$ = 0·02775 mill. The determination of the absolute velocity is of little importance, the object being rather to appreciate with exactitude and certainty the changes of rate which occur during the period of observation. *b.* If, instead of the tongue, the surface of the skin is excited with the brush, the appearances observed are very similar. The initial acceleration of the blood-stream is more easily observed by this method than by the other. *c. Direct Excitation of a Sensory Nerve.*—A frog having been curarized, the integument is divided along the outer and posterior aspect of the thigh in a line which corresponds in direction with the slender biceps muscle, or rather with the groove between the muscular mass which covers the front of the femur (*triceps femoris*) and the bulky semi-membranosus. The sciatic nerve, accompanied by the sciatic artery and vein, lies immediately underneath the biceps, between it and the semi-membranosus. In order to separate it from the vessels, it is best to bring it into view by raising the biceps on a blunt hook. Both webs having been arranged for observation under the microscope, the nerve is divided a little above the knee, and the central end laid on the copper points. The secondary coil having been placed at a considerable distance from the primary, and the eye fixed on an artery of the web of the uninjured limb, the key is opened. The same series of phenomena present themselves as before—contraction and slowing of the circulation, preceded by a much less obvious acceleration. If now the other web is brought under the microscope, it is seen that the contraction of the arteries is very inconsiderable, the acceleration is more distinct. The explanation of this is easy. The sciatic nerve being the channel by which most of the vasomotor fibres find their way to the arteries of the web, those vessels are in great measure (but not entirely) paralysed by its division. Consequently, of the three effects produced by excitation of the vasomotor centre—viz., increased vigour of the contractions of the heart, increase of arterial pressure, and contraction of the arteries—the first two only manifest themselves in acceleration of the blood-stream. In the other limb, the vasomotor nerves being intact, the phenomena present themselves in their completeness. The effect of direct and indirect excitation of the medulla on the vessels of the mesentery has as yet been imperfectly investigated. It is certain that in general the contraction of the mesenteric arteries is much less marked than of those of the web. It is often entirely absent, the only change observed during excitation being that the stream is accelerated. These facts do not indicate that these arteries are out of the control of the cerebro-spinal centres, but merely that the nerves excited are not in reflex relation with them.

52. Reflex Excitation of the Medulla Oblongata in Mammalia.—The vasomotor centre may be stimulated in the dog, rabbit, or cat, by the electrical excitation of any sensory nerve. The most convenient for the purpose is the sciatic. The requirements are the same as for an ordinary kymographic observation. If it is intended to excite the trunk of the sciatic nerve, the animal must rest on its side. It must first be rendered insensible by opium or chloral, and subsequently curarized. In order to expose the sciatic nerve, an incision must be made from a point half way between the trochanter and the promontory of the ischium towards the tendon of the biceps. Such an incision runs nearly parallel to the inner and posterior edge of the long head of the muscle just named, which edge must be found and drawn outwards. In the upper third of the thigh, the nerve lies between the biceps and the adductor magnus, further down, between the biceps and the semi-membranosus. If it is desired to stimulate the nerve near its distribution, the peronæal nerve may be found very readily in front of the ankle, on the fibular side of the common extensor of the toes. It is often called the *n. dorsalis pedis.*

Excitation of the central end of the divided sciatic or of the peronæal nerve produces effects which are indistinguishable in kind from those of direct excitation of the medulla, although the augmentation of arterial pressure and other concomitant phenomena are less considerable. In the case of the *dorsalis pedis*, however, and other nerves to be immediately referred to, there is a marked difference between the condition of the arteries in the region to which the excited afferent nerve is distributed, and those of the rest of the body.

EXPERIMENTS SHOWING THAT THE SAME DEGREE OF EXCITATION OF A SENSORY NERVE WHICH PRODUCES GENERAL CONTRACTION OF THE ARTERIES IN OTHER PARTS OF THE BODY, DIMINISHES THE TONUS OF THE ARTERIES OF THE PART TO WHICH THE EXCITED NERVE IS DISTRIBUTED.

53. (1.) **Excitation of the Nerves of the External Ear of the Rabbit.**—The ear of the rabbit derives its sensibility from two nerves, both of considerable size. One of these, the posterior auricular, approaches the surface at the back of the neck, very near the middle line, and runs forwards and outwards, under a thin covering of muscle, to the root of the ear, where it penetrates a process of cartilage, easily felt in passing the finger from the occiput outwards. By making an incision between this processs and the occipital spine, the nerve can be very easily found. The other nerve (*n. auricularis magnus, see* fig. 226) springs from the anterior branches of the second and third cervical nerves; it becomes superficial at the posterior edge of the sterno-mastoid, and then runs upwards, covered only by integument, towards the thin edge of the external ear, where it soon divides into two branches. It is most easily found at the root of the ear, just before it divides.

The animal having been curarized, the apparatus for artificial respiration is connected with the trachea, and the manometer of the kymograph with the carotid artery. The great auricular nerve is then carefully exposed, separated from the surrounding parts with the aid of two pairs of blunt forceps, and divided. The next step is to arrange the lobe of the ear in such a way that the central artery can be well seen. With this view, if sunlight is not at command, a paraffin lamp should be so placed that its light may be thrown on the ear from behind by a condensing lens, while the lobe itself is supported vertically by a suitable holder. Before beginning the experiment, the central artery should be carefully observed, attention being particularly directed to the rhythmical changes of diameter which it undergoes. Its condition having been carefully noted, and a preliminary kymographic tracing having been taken, for the purpose of preserving a record of the previous arterial pressure, the central end of the nerve is laid upon the points of the excitor, and the key opened for a couple of seconds. If no increase of arterial pressure takes place, the secondary coil, which in beginning the experiment must be distant from the primary one, is cautiously brought nearer to it until this effect is produced. As soon as this is the case, it is usually observed that the artery of the ear, instead of contracting, dilates, and that the whole lobe obviously contains more blood than it did before. Frequently, however, it happens that, notwithstanding the increase of arterial pressure, no increased vascular injection is observable. In this case, recourse must be had to the posterior auricular nerve, the excitation of the central end of which is almost certain to be followed by the effect in question. The augmentation of arterial pressure and the dilatation of the auricular artery appear to be collateral phenomena, both increasing gradually during the few seconds which succeed the commencement of electrical excitation. If care is taken neither to prolong the excitation unduly nor to use too strong currents, the reaction may be witnessed a great number of times in the same animal.

54. (2.) **Excitation of the Dorsalis Pedis.**—When the central end of the divided dorsal nerve of the foot is excited, phenomena occur of a similar nature. To enable the observer to judge of the effect, the saphenous artery must be exposed in its course down the inner side of the lower half of the thigh, as recommended in § 49, p. 241. It is then seen that during and after excitation of the central end of the divided nerve, the artery gradually dilates, subsequently regaining its former dimensions.

The general result of the preceding experiments may be expressed by saying that the afferent nerves to which they relate (in common probably with other sensory nerves) contain fibres so endowed that, when they are excited, the action of the vasomotor centre is inhibited or suspended, as regards certain regions with which the nerves in question are in close anatomical relation. In its relations to the vasomotor nervous system, the words "inhibitory" and "depressor," both of which are used by physiologists to denote the case in which arterial tonus is diminished by excitation of an afferent nerve, may be regarded as equivalent.

EXPERIMENTS RELATING TO THE EFFECTS OF DIRECT EXCITATION AND DIVISION OF THE VASOMOTOR NERVES.

When a vasomotor nerve is excited directly, the arteries of the region to which it is distributed contract. When it is divided, they become permanently larger, and remain unaffected by changes in the condition of the vasomotor centre, whether these are determined by direct or reflex excitation.

55. (1.) **Demonstration of the Vasomotor Functions of the Cervical Portion of the Sympathetic Nervous System in the Rabbit.**—In 1852, Brown-Séquard showed that when the

sympathetic nerve is divided in the neck, the central artery of the ear dilates, and the organ becomes vascular; and that when the peripheral end is excited, the same arteries contract; and in the same year he demonstrated that the former effect was dependent on paralysis, the latter on spasm of the muscular walls of the vessels.

A rabbit having been placed on the support in the prone position, about four cubic centimeters of a five per cent. solution of chloral (obtained by diluting a stronger solution with the required proportion of the ordinary solution of chloride of sodium) is gradually injected into the crural vein. [For the method of exposing the crural vein and of inserting the cannula, see § 49]. As soon as the animal is insensible, an incision is made about two inches in length parallel with the trachea, so as to expose the edge of the sterno-mastoid muscle on one side. The carotid artery is then brought into view, separated from the vagus, and drawn forward from beneath the edges of the muscle with the (fig. 203, *c*) hook, when it is seen that two small nerves, both much smaller than the vagus, are drawn forward with it, embedded in the membranous sheath (fig. 227). Of these two nerves, one, which is the smaller of the two, is the depressor—an important cardiac branch of the vagus; the other is the sympathetic. To discriminate between them, all that is necessary is to trace them both upwards. It is then seen that the depressor arises by one root from the vagus trunk, by another from the superior laryngeal; whereas the sympathetic continues its course upwards alongside of the artery. The sympathetic is also distinguishable by its grey colour. A loose ligature having been placed round the nerve, the condition of the posterior auricular artery should be carefully observed, and noted in the manner recommended in the previous paragraph. On dividing the nerve, it is seen that the artery dilates, the rhythmical movements cease, and the whole vascular network of the ear rapidly becomes injected with blood. The change in the condition of the organ is very similar, both in degree and in kind, to that observed after excitation of the central end of the auricular nerve, but differs from it in being more permanent. If after a few minutes the ears are held, one in each hand, it is felt that that of the injured side is warmer than the other. If now the peripheral end of the divided nerve is placed between the copper points and the key opened, the artery contracts and the congestion of the ear disappears.

This experiment shows conclusively that most of the spinal vasomotor nerves which are distributed to the arteries of the integument of the head, must reach their destination by passing through the superior cervical ganglion. As, however, the superior ganglion is also in direct communication with the spinal cord, the vascular paralysis is incomplete unless this communication is broken by the extirpation of the ganglion. To accomplish this, the incision must be continued upwards in the angle of the jaw (*see* fig. 227). The carotid artery and the vagus which accompanies it, having been brought into view as far upwards as the stylohyoid muscle, are drawn forwards and towards the middle line with the blunt hook by an assistant, while the sympathetic trunk is followed upwards behind the artery with the aid of two pairs of blunt forceps. The space in which the ganglion lies is crossed by the trunk of the hypoglossal nerve, and by the stylohyoid muscle. The latter should be divided. The extirpation of the ganglion is best effected with blunt-pointed scissors. After section of the sympathetic trunk in the neck, the normal condition of the ear is gradually restored; but if the ganglion is destroyed, the effect is permanent.

56. (2.) **Demonstration of the Vasomotor Functions of the Splanchnic Nerves.**—The splanchnic nerves contain (in addition to those fibres which govern the peristaltic movements of the intestine, with which we have at present no concern) sensory and vasomotor fibres. The vasomotor fibres are distributed to the arteries of the abdominal viscera. Their importance depends on the fact that these arteries receive so large a share of the systemic blood-stream (especially in the rabbit), that the resistance offered by the arterial system to the discharge of blood from the heart is largely affected by any alteration of their calibre. The sensory part of the nerve, in common with other sensory nerves, contains fibres by which the vasomotor centre is influenced. It is also, as will be seen in a future section, in reflex relation with the heart through the vagus. The splanchnic nerve in the rabbit leaves the sympathetic trunk at the 8th or 9th ganglion, passes downwards in front of the psoas major muscle, receiving branches from the other thoracic ganglia. At the level of the tenth thoracic vertebra, the two nerves lie on either side of the descending aorta, and accompany it downwards until it reaches the diaphragm, at which point the right splanchnic is further away from the vessel than the left. After entering the belly, the left splanchnic retains the same relation to the aorta as before, ending in the lower of the two cœliac ganglia, which is easily found above the left supra-renal capsule on the front of the aorta. The right nerve is more difficult to find from its lying further from the aorta, separated from it by the breadth of the *vena cava*. It ends at the level of the right supra-renal capsule, in the superior cœliac ganglion which lies in front of the vein. The splanchnic nerve may be reached either in the abdomen or in the thorax. In very exact experiments, and especially in those that relate to the functions of the afferent fibres, it is obviously desirable that these organs should not be exposed by opening the peri-

tonæal cavity; but for the purpose of demonstrating the vasomotor functions of the nerve, this precaution is unnecessary. When one of the splanchnic nerves is divided in the rabbit, the arterial pressure sinks; on electrical excitation of the divided nerve, it rises to a height which far exceeds the normal limits. Section of the other nerve is followed by further reduction, which, however, is not so considerable as that produced by division of the first. The reduction of pressure after section is attended with *increase*, the elevation of pressure after excitation with *decrease* of the frequency of the pulse. These facts are demonstrated as follows:—

A chloralized rabbit having been secured in the prone position, and one carotid connected with the kymograph, the abdominal cavity is freely opened in the linea alba. The integument is then carefully divided by a transverse incision, which extends outwards from the first incision a little below the edge of the ribs. A curved needle, of the form shown in fig. 203 *e*, guarded by the left forefinger, is then passed under the abdominal wall in the direction of the incision. Its point having been brought out about two and a half inches from the linea alba, the ligatures are tightened in such a way that the muscles are constricted at different levels. The part between the ligatures is then divided by a horizontal incision, which may be continued in the same direction without hæmorrhage. This done, the left splanchnic nerve is plainly seen running down parallel to the aorta on its left side, towards the supra-renal capsule. The space in which it lies is occupied by very loose cellular tissue covered by peritonæum, which must be broken through to get at the nerve.

Immediately after the abdominal cavity is opened—that is, before the nerves are touched—there is a very considerable rise of arterial pressure, which is accompanied with slowing of the pulse. These effects are, however, only transitory, the mercurial column sometimes sinking immediately afterwards below its original level. After division of the left splanchnic it sinks very considerably, often as much as forty millimeters (*i.e.*, more than an inch and a half). On placing the peripheral cut end between the copper points of the excitor and opening the key, the column suddenly rises. The sinking produced by section of the right nerve is comparatively inconsiderable. As it is very difficult to get at, its division may be omitted, all that is essential in the experiment being observable after section of the left.

The following numerical results are derived from one of Ludwig and Cyon's experiments:—Previous arterial pressure, 90 millimeters; after division of left splanchnic, 41 mill.; during excitation of peripheral end of divided nerve, 115 mill.; after division of right splanchnic, 31 mill. After section of both nerves, the vessels of all the abdominal viscera are seen to be dilated. The portal system is filled with blood; the small vessels of the mesentery, and those which ramify on the surface of the intestine are beautifully injected, the vessels of the kidneys are dilated, and the parenchyma is hyperæmic; all of which facts indicate, not merely that by the relaxation of the abdominal blood-vessels a large proportion of the resistance to the heart is annulled, but that a quantity of blood is, so to speak, transferred into the portal system, and thereby as completely discharged from the systemic circulation as if a great internal hæmorrhage had taken place.

PART II.—THE HEART.

SECTION V.—THE MOVEMENTS OF THE HEART.

The method of demonstrating the movements of the heart, stated in the order of their importance, are the following:—1. Exposure of the contracting heart *in situ*. 2. Application of instruments to the præcordia, for the purpose of measuring the cardiac movements of the wall of the chest. 3. Listening to the sounds of the heart. 4. Imitating the movements of the living heart by the production of similar passive movements in the dead heart.

57. Study of the Movements of the Heart in the Frog.—Before beginning the study of its movements, an adequate knowledge of the form and anatomical relations of the organ must be gained by dissection. For this purpose, the heart and great vessels should be filled with some solid substance which can be rendered fluid by warming it; such, for example, as cacao butter or the ordinary gelatin mass (*see* Chap. VI.). This must be injected by the *vena cava inferior* in sufficient quantity to fill the heart and great vessels (*see* fig. 228). It is then seen that the organ, as a whole, is egg-shaped; but is more or less flattened from side to side by a furrow which crosses the heart nearly at right angles to its axis, but inclines downwards towards the left; it is divided into an upper globular (formed of the two auricles) and a lower conical part (the ventricle). On its anterior aspect, the ventricle is continuous with a cylindrical prominence (the bulb), which projects from the anterior aspect of the right auricle, and terminates above by dividing into two arteries, the right and left aorta. Of these aortæ, which part from each other at the middle line, the left is the larger. The posterior wall of the right auricle extends backwards into a club-shaped appendage, the *sinus venosus*. This body may be described as the dilated end of the large *vena cava inferior*. It first extends vertically upwards in the middle line, in continuity with that vein, applying itself against the œsophagus behind, and opening towards the front into the right auricle, from which it is separated by a slight furrow. At the top it receives on either side the two *venæ cavæ superiores*, which, however, are relatively small. The two auricles are separated from each other by a septum, which stretches as a curtain from before backwards, between them. This curtain ends below in a crescentic margin, beneath which the two cavities communicate freely. The orifice leading from the *sinus venosus* into the right auricle is guarded by a well-marked Eustachian valve, which hangs downwards and towards the right. The auriculo-ventricular valve consists of an anterior and a posterior curtain, both of which are continuous at their edges with the auricular septum.

The mode of exposing the heart has already been described. The facts to be observed when the pericardium is opened are the following:—The series of muscular movements which are performed by the heart each time it contracts, is seen to begin at the upper end of the *vena cava inferior* and *sinus venosus*. From the sinus the peristaltic wave extends to the auricles; but it is not until the auricular contraction is complete that the ventricle suddenly draws itself together. Before this last act is accomplished, it is usually seen that the sinus venosus is full, and the auricles are already filling. In a moment they become distended and contract; transferring the blood they contain to the now empty and flaccid ventricle, which in its turn forwards it onwards to the *bulbus aortæ* and arterial system. In consequence of the fact that during the contraction of the ventricle the auricles are already filling with blood, and that the ventricle does not fill until the auricle contracts, the successive appearances presented by the heart during each cardiac period are very much as if there were a constant exchange of blood between the two great chambers into which the organ is divided, and at once suggest the notion that the auricles and ventricle dilate and contract alternately, the one seeming to contract while the other dilates, and *vice versâ*. It is easy, however, for any one who possesses the faculty of observation to satisfy himself that this is not the case; and that, while the ventricular contraction is determined by the auricular, and the auricular by that of the sinus, the last originates of itself, *i.e.*, independently of any previous movement.

The precise time between the successive acts above described may be measured by arranging a lever of the second order in such a way that while it rests near its bearings on the contracting heart, and follows its movements, its distal end inscribes those movements on the cylinder of the recording apparatus. In this way a tracing is obtained (fig. 229) in which the relaxation of the heart is marked by a rapid descent of the lever, the auricular contraction by a first ascent, the commencement of that of the ventricle by a second, and its continuance by a slow subsidence, suddenly ending in the rapid diastolic descent already mentioned. Thus in the example given, the interval between the vertical lines *a* and *b* corresponds to the auricular systole; that between *b* and *c* to the contraction of the ventricle—so that the auricles are in diastole from *b* to *a*, the ventricles from *c* to *b*.

58. **Study of the Movements of the Heart in Mammalia.**—For this purpose a rabbit must be completely chloralized. The trachea having been connected with the apparatus for artificial respiration, and the frequency and quantity of the inflations carefully regulated, the chest is opened in the manner already indicated in § 49. The facts to be studied are the following:—*a*. At the beginning of the period of relaxation, the heart is so flaccid that it obeys the law of gravitation and is consequently flattened from side to side, just as we usually see it in the dead body. It does not follow from this observation that the relaxed heart has the same form when enclosed in the thorax, but on other grounds it probably is so. For its form within the chest when in the flaccid condition is manifestly determined partly by gravity, partly by the shape of the space in which it is contained; and inasmuch as the space is a wedge-shaped one, bounded anteriorly by the sternum and ribs, posteriorly by the diaphragm, but virtually unlimited towards either side, we may be quite sure that the organ is at least as much flattened antero-posteriorly in the natural state, as it is seen to be when the chest is open. *b*. During the remainder of the diastole the ventricles are still flaccid and perfectly passive, but the conditions are changed. While gradually filling with blood, they go through those changes of form which are exhibited by a bladder contained in a basin when it is gradually filled with water. *c*. At the end of diastole follows a very short period, during which, although the ventricles are still soft, active muscular movements can be observed. This is known as the præ-systolic period. Systole has in reality begun; but the auriculo-ventricular valves not having yet had time to close, the ventricular contraction is unresisted. The heart, like any other muscle, so long as it contracts without opposition, is soft. *d*. The moment that the valves close, the heart hardens and becomes globular, slightly twisting round its axis, while the apex is thrown forward, and at the same time approaches the base. If at the moment of ventricular hardening the attention is fixed on the aorta, that great artery is seen to undergo the same changes of form which we have already studied in the arterial pulse—changes due partly to lateral expansion, *i.e.*, increase of diameter; partly

to axial expansion, *i.e.*, increase of length. The "locomotive" movement, which results from the axial expansion of the aorta, has its influence on the heart; for it compensates for the axial shortening which occurs when the *heart gathers itself up into a globe* to overcome the arterial resistance which is opposed to it at the moment that it begins to force its contents into the already distended arteries.

In the preceding paragraphs the attention of the student has been directed entirely to the arterial side of the heart, *i.e.*, to the movements of the ventricles and great arterial trunks. These having been mastered, he must next observe those of the auricles, with special reference to the order of time in which they occur.

At the commencement of the period of ventricular relaxation the whole heart is flaccid. The duration of this period varies inversely as the frequency of the pulse, so that no general statement can be made with respect to it. As long as it lasts, blood enters the auricles from the systemic and pulmonary veins. At a moment which anticipates the hardening of the ventricles (in the rabbit) by something like a fifth of a second, the auricles harden, while the ventricles, which have already received a certain quantity of blood through the open auriculo-ventricular orifices, fill much more rapidly. This hardening of the auricles is not, however, to be compared either in vigour or suddenness to that of the ventricles; it does not affect the whole auricle at once, but rather seems to spread from the venæ cavæ towards the ventricles as a wave of contraction. While the auricle is still contracting, the preparatory "præ-systolic" movements begin in the ventricles, culminating, as already described, in the ventricular shock, or heart pulse.

To complete the study of the movements of the heart *in situ*, they should be observed under various abnormal conditions, *e.g.*, under the influence of section and excitation of the vagi, in dyspnœa and after hæmorrhage. The appearances then seen will be referred to under the proper heads.

59. The Cardiac Impulse.—It has been already stated that the ventricular part of the heart is contained, both in man and in the lower mammalia, in a somewhat wedge-shaped space, the posterior wall of which formed by the diaphragm is more or less resistant. Consequently, when the ventricles suddenly harden and become globular, they knock against the wall of the chest with more or less violence. This knock is called the cardiac impulse. It is precisely coincident with the complete closure of the auriculo-ventricular valves, and determines the bursting open of the sigmoid valves. If the base of the heart, *i.e.*, the roots of the great arteries, were fixed, the shortening of the ventricular axis, which, as we have seen, occurs at the moment of hardening, would determine a withdrawal or retraction of the apex from the position occupied by it in diastole. As, however, this shortening is attended with lengthening of the aorta, its retractive effect is more or less neutralized, so that the seat of impulse—in other words, the centre towards which the muscular mass of the ventricles draws itself together — is not far from the position occupied by the apex of the heart when in a state of relaxation. This can be demonstrated both in man and in the lower animals. In a rabbit or dog rendered insensible by opium or chloral, a number of long slender needles are introduced into the heart in the following positions:—No. 1 is inserted vertically into the ventricle at the point at which its knock can be felt by the finger most distinctly. From this point a line is drawn upwards and inwards towards the root of the aorta, along which Nos. 2, 3, and 4 are inserted in a similar manner in the intercostal spaces. In like manner, Nos. 5 and 6 are inserted at equal distances on either side of the impulse in the same intercostal space. The movements executed by these several needles differ according to their relation to the central one, No. 1, which, although it is affected by the ascent and descent of the diaphragm, is indifferent as regards the heart. Of the series Nos. 2, 3, and 4, the free end of each performs an instantaneous upward movement, the extent of which is in proportion to its distance from No. 1; and finally, Nos. 5 and 6 oscillate more or less horizontally, their free ends receding from each other, as well as from No. 1, at the moment of the impulse. From these facts we learn that, whereas that part of the ventricular mass which knocks against the chest is nearly stationary, the base of the heart moves downwards and to the left at the moment of the ventricular hardening, *i.e.*, of the aortic pulse; and that the other parts of the ventricles are drawn towards the impulse in a degree proportional to their distance from it.

In man, the same facts are demonstrated with the aid of the cardiograph. The word cardiograph has been applied by various writers to a variety of instruments, which differ from each other both in their form and in the principles on which they are constructed, but agree in the purpose which they are intended to fulfil. This purpose is the recording of the cardiac movements of the wall of the chest by the graphic method.

60. **The Cardiograph.**—The cardiograph I use is shown in fig. 230. Its most important part is a hollow disk, the rim and back of which are of brass; the front is of thin india-rubber membrane. This disk is called a tympanum. To the brass back a flat steel spring is screwed, which is bent twice at right angles in the same direction, in such a way that it overhangs the india-rubber membrane. The extremity of this spring, which is exactly opposite the centre of the face of the tympanum, is perforated by a steel screw, the point of which rests on the membrane, while its

head is surmounted by an ivory knob. The tympanum is further provided with three adjusting screws, by which, when in use, it rests on the wall of the chest, with its face parallel to the surface, and can be approximated or withdrawn at will. It is evident that when the screws are so adjusted that the spring presses on the chest, whatever movements of expansion or retraction are made by the surface to which it is applied are communicated to it, and by it to the india-rubber membrane with which its point is in contact. The cavity of the disk communicates by a vulcanized india-rubber tube with a second tympanum, represented in fig. 231, in such a way that the two tympana and the tube enclose an air-tight cavity. The result of this arrangement is, that whatever movement is performed by the first is simultaneously reproduced, but in the reverse direction, by the second. If the tympana are of equal area, the extents of the primary and secondary movements are equal. When, as is usually the case, the areas are unequal, the extent of movement is approximately inversely proportional to the areas. The movement of the second tympanum is magnified and inscribed on the registering cylinder by a lever in the manner explained in a previous paragraph. By this apparatus a tracing is obtained, which is an exact representation of the movements of the surface against which the spring is applied, so that, if the instrument is graduated, it may be used not only for the purpose of estimating the relative duration of those movements, but for measuring their extent.

For the purpose of studying the cardiac impulse in the human chest, the subject should be allowed to rest supine on a flat surface, with his head on a pillow. The impulse is sought for in the normal position, *i.e.*, in the space between the fifth and sixth ribs, about half an inch nearer the sternum than the mammary line (the line which passes vertically through the nipple). On applying the cardiograph in this position, with the ivory knob pressing against the seat of impulse, a tracing is always obtained which has the general characters exhibited in fig. 232*a*, in which the moment of hardening is indicated by a sudden ascent of the lever, and the end of the ventricular systole by an equally marked, but not so sudden, descent. If now the cardiograph is shifted towards the sternum, the character of the tracing is entirely altered. (*See* fig. 232*b*.) The ventricular hardening is still, indeed, indicated by a jerk upwards of the lever; but this is immediately succeeded by a descent of such a character as to afford evidence that at the point investigated, the thoracic wall, instead of bulging, is retracted during the systolic effort. This phenomenon, which is well known to pathologists, being so marked in some conditions of disease that it is easily appreciated by the unaided hand or eye, has been called the "negative impulse." It means that the heart, which when gradually filling with blood applies itself to the whole præcordia, gathers itself from all directions towards the centre of impulse (in bed-side language, commonly miscalled the apex). If the cardiographic tracing of the impulse is compared with that obtained manometrically by a method to be immediately described, it is obvious that the two correspond with each other very closely; so that we are perfectly safe in assuming, as has been done above, that the ascent denotes the beginning, the descent the end of the ventricular effort. We can thus determine with the greatest precision the moment at which the mitral and tricuspid valves close. The moment of the closure of the arterial valves is not so certain, for it does not coincide with the end of the systole. It is sometimes marked by an up-and-down movement of the lever, due to the vibration into which the chest wall is thrown at the moment that the curtains of the aortic valve come together. The auricular contraction is often indicated by a slight elevation, which precedes the impulse by a distinct interval.

61. **Investigation of the Sounds of the Heart.**—The sounds of the heart can be studied both in man and in the lower animals. The first or dull sound coincides with the hardening of the ventricles, the complete closure of the auriculo-ventricular valves, and the bursting open of the arterial orifices.

It is caused principally by the sudden distension of the ventricles, but can be proved experimentally to be also in part of the same nature with the noise made by all muscles in the act of contracting against a resistance. The second or sharp sound is coincident with and caused by the closure of the sigmoid valves. This is proved by the observation that if the valve is injured or prevented from closing by mechanical means, the sound is no longer heard. In studying the sounds of the heart in the lower animals, particularly in the dog, the student of medicine should direct his attention specially to the modifications of the sounds under known conditions: *e. g.*, in dyspnœa, when the heart is distended with blood; after hæmorrhage, when the ventricles are insufficiently filled in diastole; after section of the vagi, when the frequency of the contractions is so great that the aortic valves have not even time to close; or under the various conditions in which these nerves are directly or indirectly excited. From all these modifications, the efficient causes of which are known and understood, lessons may be learnt which may be applied directly at the bed-side as aids in the interpretation of analogous phenomena when they present themselves in man.

62. **Study of the Action of the Valves in the Dead Heart.**—Although this method forms no exception to the general rule that little can be learnt in physiology by teleological inferences from the properties of dead organs or tissues, it is yet of great value to the student for the purpose of illustrating the purely

mechanical part of the action of the heart. The heart of any mammalian animal may be used, that of the pig being most suitable. The simplest method of imitating the conditions which actually exist in the circulation, consists in bringing one or other of the ventricles into communication with a reservoir placed at a sufficient height above it by means of two flexible tubes. The most convenient form to be given to the reservoir is that of a glass funnel, the stem of which communicates by one of the flexible tubes with the aorta. The other tube ends in a large glass cannula, which is securely tied into the ventricle near its apex; its opposite end is fitted to a glass syphon, the short leg of which dips into a funnel; the tube is guarded by a clip. The funnel and syphon having been filled with water and the clip closed, the apparatus is ready. On opening the clip, water flows into the right ventricle and distends it; on closing it and compressing the ventricle with the hand, its contents are forced upwards through the aorta into the funnel, while the tricuspid valve is distended. To observe the action of that valve, all that is necessary is to cut away part of the wall of the right auricle. It is then seen that when the ventricle is squeezed, the liquid contained in it tends to rush outwards by the auriculo-ventricular opening, carrying the valve with it. In a moment the curtains become distended, meeting by their borders so as to form a tense membranous dome, which projects into the auricle. The time which intervenes between the commencement of the compression and the tightening of the valve varies according to the vigour of the contractions, the quantity of blood contained in the ventricle, and the previous position of the valve, but must always be appreciable. It corresponds to the præ-systolic period previously referred to. All these facts are learnt much more impressively by introducing the index finger into the right auricle of a large animal. In the horse this can be done easily by an opening of such size that the finger is tightly grasped by it. The valve bulges out as a tense membranous dome into the auricle at the moment of auricular contraction. In observing the action of the tricuspid valve in the dead heart, it is important to notice what are the conditions which render the valve incompetent, *i.e.*, prevent it from closing completely. The most important of these conditions is over-distension of the ventricle, by which the ostium becomes too large to be covered by the valve. When this occurs during life, the phenomenon known as the venous pulse presents itself. The right ventricle being still in communication with the venous system at the moment that it hardens, blood is injected by it backwards. When (in the human subject) this condition is permanent, it leads first to dilatation of the great veins, and, secondly, to similar incompetence of the vein-valves nearest the heart. In such persons two large swellings are seen on either side of the neck—the distended jugular veins—which pulsate nearly synchronously with the heart.

Section VI.—Endocardial Pressure.

By this term is understood the pressure exercised by the blood contained in the heart, against its internal surface. It can be measured in the frog and in mammalia.

63. Investigation of the Endocardial Pressure in the Heart of the Frog under various Conditions.—In the frog the action of the heart is maintained unimpaired after the separation of the organ from the cerebro-spinal nervous centres. It is not even necessary that it should be supplied with blood. Serum (if perfectly fresh) of another animal may be substituted for it, without apparently affecting either the vigour or regularity of the cardiac contractions. These two facts render it possible to use the heart of the frog for the solution of a number of problems, in reference to which it is desirable to investigate the mechanical functions of the heart independently of the influence of the nervous system.

The method of preparing the heart for such experiments is that first employed by Dr. Coats, of Glasgow, in an investigation relating to the mechanical work done by the heart in a given time, in Ludwig's laboratory. It has been since used with various modifications by Bowditch, Brunton, Blasius, and others. The brain and spinal cord having been destroyed by the introduction of a needle, the body of the frog is cut across below the liver. The sternum with the anterior extremities are removed, great care being taken to reserve on one side a large flap of skin which may be used as a cover for the nerves and the heart. The heart is then freed of its pericardium, and the little serous ligament by which it is connected with the posterior surface of that membrane is ligatured and divided. The next step is to tie one branch of the aorta, and then to pass a cannula through the other and the bulb into the ventricle. The suspensory ligaments of the liver are then severed so as to expose the vena cava inferior. A ligature is passed round that vessel, which is then slit open so as allow a large cannula to pass into the right auricle. The cannula having been secured, the liver and lungs are removed, the stomach is severed through the middle, and a stout glass rod, tapering at either end, is passed from the mouth down the œsophagus. This rod should be as large as possible, as the stretching of the parts between the heart and the spinal column which is thus produced materially facilitates their satisfactory exposure. The end of the glass rod which projects from the mouth must then be fixed in a support, and the tube which is inserted in the right auricle be fitted with a flexible tube and connected with a glass reservoir (for which purpose one of the patent syphon inkstands does best) filled with reddish rabbit serum. The aorta is in like manner connected with a manometer of the form indicated in fig. 233, from which the general arrangement of the heart, reservoir, and manometer will also be best understood.

The heart is charged with serum and brought into action by filling the reservoir. From thence the liquid fills the right auricle, passes therefrom to the ventricle, and is discharged by it into the manometer. As soon as it is seen that no more air bubbles pass through the proximal limb of the manometer (the upper end of which is connected with a flexible tube for the purpose of conveying the liquid pumped by the heart to a suitable receptacle), the apparatus is ready. The mode of experiment may be varied according as it is intended merely to measure the variations of endocardial pressure which occur during a cardiac period, or to observe the modifications which that pressure undergoes under different mechanical conditions.

64. *a. Variations of Endocardial Pressure which occur during each Cardiac Period.*—To observe these, the heart must communicate exclusively with the manometer, the proximal limb of which with the tube leading to it from the ventricle, and the ventricle itself, must form one cavity filled with serum, and closed towards the auricles by the valve, and in the opposite direction by the mercurial column and a clip, by which the tube connected with the upper end of the proximal limb is guarded. The manometer should be at such a height that when the pressure is greatest the top of the proximal column is at the same level as the heart; and the quantity of mercury it contains must be adjusted, by addition or subtraction, with the aid of a capillary pipette, so that when the heart is in diastole the distal column is still about a millimeter higher than the other. The reservoir for the supply of serum must now be placed at such a height above the heart that the pressure in the auricle is equal to that existing during diastole in the ventricle; and inasmuch as this has been already arranged at a millimeter of mercury, the height of the venous column of serum must be about half an inch = 12 millimeters, the specific gravity of mercury being about twelve times that of serum. In the distal column of the manometer is a glass piston, the upper end of which bears a horizontal arm arranged in the same way as that which bears the writing pencil in the ordinary kymograph—the main differences being that in this case the manometer is much smaller, and that, in order to avoid friction, the tracing is recorded, as in the sphygmograph, on glazed paper, blackened by passing it over the flame of a paraffin lamp. The record so obtained is shewn in fig. 234. On account of the relative slowness of the movements and the inconsiderable lumen of the manometer, the curve is very little modified by the

oscillation proper to the mercurial column, and is therefore a true representation of the succession of changes of pressure which take place in the ventricle. We learn from it that in the frog the pressure exercised by the ventricle on the blood it contains arrives at its acme somewhat gradually, and persists for an appreciable period; and that when the heart relaxes, the subsidence of pressure is at first extremely rapid, but subsequently somewhat more gradual. The rate of movement of the paper being 40 centimeters per minute, the duration of each systole can be easily measured.

65. *b. Modifications of the Endocardial Pressure Curve under various Conditions.*—For the purpose of investigating the influence of various mechanical conditions on the action of the heart, and particularly of changes in the relation of the pressure in the veins and that in the arteries, the apparatus must be so modified that the ventricle, instead of communicating exclusively with the manometer, pumps the liquid, constantly supplied to it from the venous reservoir, along a tube or system of tubes representing the arterial system. To fulfil these conditions, all that is necessary is, (1) to insert the arterial cannula, not in the bulb, but in the left aorta (the right being tied), so as not to interfere with the play of the aortic valve; and (2) to join to the proximal limb of the gauge an india-rubber tube, dilated near the junction into an elastic bulb, and ending in a nearly capillary beak of glass, the purpose of the latter being to furnish the required resistance, that of the former to render the discharge as nearly equable as possible—in short, to replace the elasticity of the arteries.

The advantage of this arrangement does not lie in the circumstance that the mode of action of the heart is more natural, for it makes little difference to that organ whether the liquid it discharges at one contraction returns to it during the next relaxation or is pumped forwards, provided that the pressures to which it is subjected are the same in systole as in diastole. It is rather that when the heart is so arranged that liquid is pumped through it continuously, the observer has it in his power to modify the arterial pressure (by altering the resistance) without modifying the venous pressure, and vice versâ, and so to reproduce conditions which actually exist and exercise a most important influence in the living body.

It is obvious that if the pressure on the venous side of the heart is nil, no progressive movement will occur, whatever may be the resistance in the arteries; and, on the other hand, that if the pressures on the two sides of the heart are equal, there must also be no movement, for the auriculo-ventricular valve remaining open, the heart would act as in the previous experiment, receiving back again in diastole whatever liquid it discharged during systole. between these two extremes, that of equality of venous and arterial pressures and that of total want of pressure in the auricles, a mean relation exists which is most advantageous to efficient action, and cannot be departed from in either direction without impairment of effect. The existence of this *ratio of greatest efficiency* has been lately demonstrated experimentally by Blasius;* and it has been found, first, that for every value of arterial resistance, it is possible by successive trials to ascertain what venous pressure enables the heart to contract with the greatest effect; and, secondly, that for every heart there is a certain value of arterial resistance which is most advantageous. The mean result of numerous observations is, that the frog's heart (rana esculenta) does most work when it is opposed by an arterial pressure of about 35 millimeters of mercury. If the resistance is greater than this, the heart becomes over-distended, and its valves incompetent.

66. Application of the preceding Methods to the Investigation of the Problem of the Mechanical Work done by the Heart in a given Time.—In the preceding paragraph, the expressions, mechanical "effect" of the heart's contractions, and "work" done by the heart, have been used without explanation. Before proceeding further, it is necessary to define them. The work done by the heart in any given time is equal to the product of the aortic pressure and the quantity of blood which passes through the aortic orifice in the same time. To illustrate this, it is necessary to revert to the experiment described in § 46, in which the circulation is maintained artificially in the frog by substituting for the heart a column of serum of sufficient height. In this case, so long as the height of the column remains unaltered, the work done in carrying on the circulation truly represents that of the heart. If it is allowed to diminish, the rate of flow diminishes with it. To maintain constancy in the circulation, the liquid discharged by the sinus venosus must be constantly replaced in the funnel as it flows out. The work which is expended in doing this per minute is the work by which the circulation is carried on. Thus, supposing the height of the column of serum to be 400 millimeters, and that it is found that the level of the liquid in the funnel begins to subside when not supplied at such a rate that the weight of serum flowing through the aorta during one second is equal to one-fifth of a gramme, then the force expended per second would be that required to raise one-fifth of a gramme 400 millimeters, *i.e.*, one gramme to the height of a meter in 12·5 seconds, or 0·08 grammes to the same height in one second; and this result has been arrived at in accordance with the proposition with which we started, by multiplying the aortic pressure (expressed in the height of a column of blood corresponding to it) by the quantity discharged in the given time.

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* *Am Frosch-Herzen angestellte Versuche über die Herz-Arbeit, &c.* Fick's *Arbeiten*, Würzburg, 1872, p. 1.

If exact information were attainable as to the quantity which the heart actually discharges at a stroke, it would be possible to measure the quantity of work done by the heart in the maintenance of the circulation in a mammalian animal, and inferentially in man; but inasmuch as no such method at present exists, no estimate can be given which possesses even approximate value. In the frog, however, a reliable estimate can be made by the methods described in § 63, whichever form of experiment is employed. Thus, when the heart communicates exclusively with the manometer, the work which the heart is made to do is to raise whatever quantity of mercury is contained in the manometer between the level at which it stands during diastole and that to which it rises in systole, to the mean height $\frac{h}{2}$, where h denotes the difference in millimeters of the two levels. For evidently, of the whole number of particles of mercury in the distal column, the surface of which is caused to rise h millimeters above the surface in the proximal column, it is only the top particles which are raised h millimeters above the level of the proximal column; those in the exact middle are raised only half h; those above and below, less or more in proportion to their distance from the middle; so that the mean elevation is half h. The weight is easily known if we know the area, *i.e.*, lumen, of the tube, and the specific gravity of the mercury. If we designate the former as a and the latter as s, we have the weight lifted by the heart in each contraction to the height $\frac{h}{2}$, expressed by $a \cdot s\ h$, and the work done (that is, the product of the weight lifted and the height to which it is lifted) $\frac{ash}{2}{}^{2}$. If it is desired to obtain perfectly accurate results, a manometer must be used of which the area of the surface of the mercury in the proximal limb is relatively very large. In the other form of experiment, § 64, *i.e.*, when a continuous current of serum is pumped by the heart along a tube representing an arterial system, the problem assumes a somewhat different form. The rate of flow through the tube must be first ascertained by measuring the discharge from its terminal orifice. This being known, the answer to the question is arrived at by considering what height of column of serum would, if substituted for the heart, be sufficient to determine the same rate of efflux. This can be learnt most accurately by a comparative experiment; it can be deduced approximately from the measurement of the mean pressure actually existing in the aorta. Here, as before, the mechanical work done by the heart is the work which would be required to raise the quantity of serum discharged per second to the height corresponding to the pressure, *i.e.*, to a height something like twelve times that indicated by the mercurial manometer.

67. Investigation of the Endocardial Pressure in Mammalia.—As this mode of investigation can only be practised on animals of large size, and has already perhaps yielded all the results which can be expected from it, it will be sufficient to give a cursory account of it here, referring the reader to the papers of its author, Professor Chauveau, for detailed information. The method consists in lodging in one or other of the cavities of the heart of an animal, an india-rubber bag, or ampulla, which communicates by a long narrow tube with a manometer. The introduction of the instrument in question (which has received the name of cardiac sound) into the right cavities through the external jugular vein is perfectly easy, and can be effected in the horse, as I can testify from my own observation, without occasioning the animal the slightest suffering or even inconvenience—a fact easily enough understood when we reflect that the internal surface of the vascular system is not supplied with sensory nerves. The ampulla does not come in contact with the surface of the heart. The left ventricle is reached through the carotid artery with somewhat greater difficulty. The left auricle is of course inaccessible.

The most important results have been obtained by a cardiac sound so constructed that the variations of pressure can be recorded in the right auricle and ventricle simultaneously. By means of this instrument, M. Chauveau has been able to demonstrate the order of succession of the movements of the heart, and the intervals of time which separate them from each other, with an exactitude which would have been otherwise unattainable. Thus he has shewn that in the horse the interval between the hardening of the auricle and that of the ventricle is just about a tenth of a second, and that the duration of the ventricular systole is about three-tenths, whatever be the number of contractions per minute; so that frequency of the pulse depends not on the time taken by the heart to accomplish each contraction, but on the interval of relaxation which separates one systole from its successor. (*See* fig. 235.)

Chauveau found the systolic pressure in the horse to be about 128 millimeters in the left ventricle, and 25 millimeters in the right. These numbers express the *relative* values of the mechanical work done by the two ventricles. The absolute values, as has been already stated, are unknown, from the impossibility of determining the quantity of blood which flows through the heart in a given time.

SECTION VII.—INTRINSIC NERVOUS SYSTEM OF THE HEART.

Nothing is as yet known either as to the anatomical distribution of nervous elements in the hearts of mammalia, or as to the functions which they perform. In the frog, both have been the subject of minute and repeated investigation. We have already had frequent occasion to observe that the frog's heart continues to beat after its removal from the body, and that this rhythmical movement often goes on for hours or even for days, under favourable circumstances. From this it is evident that its maintenance is dependent on conditions which are contained within the heart itself.

68. Proof that the Ganglion Cells contained in the Heart are the Springs of its Automatic Movement.—It is objected by some physiologists that the rhythmical contractions go on not merely in the whole heart when deprived of blood and severed from the cerebro-spinal nervous system, but also in mere fragments of the muscular substance which cannot be admitted to contain ganglion cells. The answer lies in the results of the following experiments:—

The heart of a frog just removed from the body is placed in a watch-glass containing serum, or three-fourths per cent. saline solution, in which it will continue to pulsate for many hours. Small portions of muscular substance are then taken either from the *sinus venosus*, the auricles, or the ventricle, and observed in a drop or two of the indifferent liquid, under a low power. It is then seen that portions taken from the sinus, the auricles, or that part of the ventricle which is in the immediate neighbourhood of the auriculo-ventricular constriction, pulsate rhythmically, but that similar portions taken from the ventricle near the apex do not pulsate. The pulsating bits may be further divided with sharp scissors under the dissecting microscope, until preparations are obtained which consist of only a few muscular fibres. Many of these still contract rhythmically, each fibre becoming shorter and thicker at each contraction, but not losing its rectilinear contour. If now the pulsating and non-pulsating shreds are submitted to microscopical examination, it will be found that, whereas ganglion cells cannot be seen in the latter, they exist as a rule in the former. In the recent state, indeed, it is quite impossible to demonstrate their presence in either case, but they can be detected after preparation with chloride of gold in the manner directed in Chap. IV.

69. Description of the Intrinsic Nervous System of the Heart of the Frog.—The heart of the frog is not known to receive nerves from any source excepting the vagus. The cardiac branches of this nerve, as they enter the heart (*see* § 73), apply themselves to the superior vena cava close to its origin, and then, after giving numerous branches beset with ganglionic cells to the *sinus venosus*, the two nerves combine to form a plexus at the upper part of the septum, between the auricles. From this plexus two filaments descend, the smaller along the anterior edge of the septum, the larger along the posterior. On approaching the auriculo-ventricular orifice, each of them exhibits a distinct bulging (Bidder's ganglia), from which radiating streaks may be seen to spread towards the ventricle.

So long as the nerves are still outside of the heart they do not contain any ganglion cells, nor give off any branches; but as they approach the plexus they become beset with cells, and give off numerous filaments to the *sinus venosus*. The two branches (anterior and posterior) have no special relation to the two *rami cardiaci* from which they in common originate, although Bidder finds that the anterior contains more fibres from the right side, the posterior from the left. In their course, both filaments give off branches, which ramify in the septum or pass into the wall of the auricles. In order to see these nerves, the heart must be exposed by opening the pericardium. Its point must then be drawn upwards, the two aortæ divided, and the ligamentous shred which connects it with the posterior surface of the pericardium cut through. The two *venæ cavæ* must then be divided as far from the heart as possible, and the heart removed. If the organ is now stretched on a wax plate by means of fine pins stuck into the venæ cavæ, one into the vena cava inferior, and one into each vena cava superior, and examined under water, the two vagi (*rami cardiaci*) can be seen where they are in relation with the vena cava superior. If now the apex is drawn to the right and fixed by a fourth pin, the side of the left auricle is exposed, and may be slit open with fine scissors, so as to bring into view the septum, which must then be cleared of the outer wall of the auricle by careful dissection. Fig. 236 shews the appearance of the septum prepared in this way.

70. Demonstration of the Special Functions of the Ganglia. 1. *Stannius' Experiment*. The heart of a frog having been exposed in the usual way, a stout glass rod is introduced into the œsophagus. All the other organs may now be removed in the manner directed in § 63, care being taken to avoid interfering with the venæ cavæ. The glass rod having now been fixed horizontally on the table, and the œsophagus secured by pins stuck through it into the table so as to prevent it from slipping on the rod, the apex of the heart is seized with blunt forceps and drawn forwards and to the right. A silk ligature is then passed, with the aid of the needle shown in fig. 203*b*, between the vena cava inferior and the ventricle, and between the venæ cavæ superiores and the right auricle, in such a position that when it is tightened it will grasp the line of junction between the sinus venosus and the right auricle. The ligature having been looped by an assistant and carefully adjusted in the proper position, the heart is left to itself. As soon as it is seen that it is contracting regularly, the ligature is tightened. After one or two beats, the heart stops in a state of relaxation. The pulsations of the sinus, however, continue at the same rate as before. After a time the ventricle also begins to beat; but on comparing its rhythm with that of the sinus, it is seen that they do not agree.

2. In another heart, prepared in the same manner, the sinus is cut off from the right auricle, the line of amputation corresponding with that of the ligature in 1. In doing this, the heart must be drawn forwards with the forceps by its apex as above directed. The result is more striking when the scissors used are not very sharp.

3. If in either of the above experiments the ventricle is cut off from the auricles immediately after the ligature or amputation, as the case may be, it begins to beat again at once.

4. In a third heart, the line of ligature, *i.e.*, the junction between the *sinus venosus* and the right auricle, is excited by the induced current. For this purpose Du Bois Reymond's induction apparatus is used. The points of the excitor must be very close to each other. The effect resembles that of the ligature. If the electrodes, instead of being placed so as to include the sinus, are applied to the auricles, no effect is produced.

5. In another animal, $\frac{1}{1000}$ of a grain of atropin (or less) is injected underneath the skin. After a few minutes the heart is removed, and experiment 4 is repeated. The electrical excitation produces no effect, the ganglion of the septa being paralyzed. Experiment 1 is then repeated. The heart stops as before.

All the preceding results can be obtained in the separated heart. The method recommended facilitates the manipulation without in the slightest degree impairing the value of the results. Stannius' experiment admits of two different explanations, which are not, however, inconsistent with each other:

1. The arrest of the heart may be regarded as a result of the excitation of the ganglion of the septum, *i.e.*, the mechanical irritation of that part produced by the scissors or ligature; in other words, as an effect of the same nature as that produced in experiment 4, where that centre is subjected directly to electrical stimulation; or,

2. It is dependent on the severance of the sinus venosus from the rest of the heart. In this case it must be regarded as of a different nature from the arrest produced by electrical excitation.

If it were not for experiment 5, we should be inclined to adopt the former of these views; for it is very easy to imagine that it is not likely to make much difference whether we squeeze the ganglion with a ligature, nip it between the blades of a pair of scissors, or excite it by Faradaic electricity. Indeed, any one who compares the two results—the arrest of the heart by electrical excitation of the sinus on the one hand, and that produced by ligature across the upper part of the auricles on the other—would probably at once decide on their identity. By previously subjecting the heart to the influence of atropin, we are enabled to demonstrate that such a conclusion would be erroneous; for if the effect of ligature were of the same nature, it would be counteracted by the same agency.

In order to explain the phenomena, it is necessary to assume, what has not yet been proved anatomically, namely, that the venous sinus contains an *automatic motor centre.* By this term we understand (in accordance with the general notions entertained as to rhythmical action) a ganglionic centre, in which energy tends to accumulate and discharge itself in the form of motion at regular intervals, the length of which varies (*a*) with the resistance to the discharge, and (*b*) with the rapidity of accumulation.

The physiological ground for this assumption of the existence of a motor centre in the sinus venosus is, first, that the succession of acts which make up a cardiac contraction commences distinctly in the sinus, and that it is the only part of the heart which contracts independently, *i.e.*, without being affected by the action of any other part of the organ; and, secondly, that electrical stimulation of the sinus induces increased frequency of the contractions of the whole organ. Admitting the existence of such a centre, and assuming also that the ganglion of the vagus, situated, as we have seen it to be, close to the line of ligature or amputation on the auricular side of it, has the power of *inhibiting, i.e.,* increasing the resistance to the discharges from that centre, and further that it exercises a similar inhibitory influence on the motor ganglia at the base of the ventricle, we are enabled to harmonize the experimental results completely thus:—In the ligature and amputation experiments, the heart stops for two reasons: first, because the ventricle is separated from the motor centre; and, secondly, because, by the pressure or mechanical irritation of the ligature or blunt scissors, the vagus ganglion is excited. In electrical excitation, on the other hand, the second of these effects is produced without the first; consequently, when under the influence of atropin, the vagus ganglion is paralyzed—the influence of ligature and amputation, in so far as they are dependent on severance of the sinus from the rest of the heart, are unaltered, but electrical excitation is without result.

On this subject the student will do well to consult the original papers, the references to which are as follows:—As regards the anatomy of the ganglia, the most important paper is that of Bidder, in Müller's Archiv, 1852, p. 163; as regards their functions, Stannius (Müller's Archiv, 1852, p. 85), Nawrocki (Der Stanniusche Herzversuch, Heidenhain's Studien, 1861, p. 110), and Schmiedeberg (Untersuch. über einige Giftwirkungen am Froschherzen. Ludwig's Arbeiten, 1871, p. 41).

71. Study of the Influence of Changes of Temperature on the Heart.—(*a*) *In the Frog.* Inasmuch as the influence of temperature is obviously dependent on the intrinsic nervous system, the present is the proper time for considering it. The modes of investigation are the same as those already described in the section on endocardiac pressure. Exact and extended researches have been made by both of the methods there given, the first having been employed by Cyon, the second by Blasius. Of the two, the latter is preferable, on account of the greater ease with which the work done can be measured. The general result is, firstly, that the quantity of mechanical work which can be done by the heart in a given time increases with the temperature up to a certain point (about 20° C., but it differs in different animals, and no doubt also at different seasons), so that it may be doubled or trebled by a gradual rise from ordinary winter temperature to that of summer; and, secondly, that under the same circumstances the frequency of the contractions increases in much greater proportion than the mechanical effect. Hence it results that although the total quantity of work done in a given time is *less* at lower temperatures than at higher, the effect of each individual contraction is much greater.

If it is desired merely to observe the effect of changes of temperature on the frequency of the pulse, much simpler apparatus will answer the purpose. Either the whole heart may be used or a part of it. In the former case, the organ having been removed from the body is suspended by a thread attached to the aorta in the interior of a tolerably wide test tube furnished with a cork, through the centre of which the thread is drawn. At the bottom of the tube there is a bit of blotting-paper, soaked with water. The "moist chamber" so prepared is immersed vertically in a test tube filled with cold water, which also contains a thermometer. The water in the beaker is then very gradually warmed, while its temperature and the frequency of the contractions of the heart are noted from time to time. It is then seen that the frequency gradually increases up to about 34° C., above which the contractions become irregular, and are difficult to count with exactitude, until at last the condition known as "heat rigor" (with reference to which see Chapter XX.) supervenes. Similar observations may be made with respect to portions of the heart, as, *e.g.*, the base of the ventricle or the sinus venosus. For this purpose it is convenient to place the fragment on a cover glass in a drop of serum, and invert it over the chamber of Stricker's warm stage.

72. (*b*) *In Mammalia.*—From the observation of the very remarkable effects which diminution and increase of the internal temperature of the body respectively produce, the one in diminishing, the other in increasing, the frequency of the pulse in rabbits and dogs,

it seems probable that the mammalian heart is more sensitive to temperature changes than that of the amphibia. As, however, it is not possible to eliminate the influence of the central nervous system, this cannot be proved experimentally.

SECTION VIII.—THE INHIBITORY NERVES OF THE HEART.

73. **1. Demonstration of the Influence of the Vagus Nerve on the Heart in the Frog.**—*Description of the Vagus Nerve.*—The vagus nerve originates in the frog from the posterior aspect of the medulla oblongata by three or four roots, the lowest (analogous to the spinal accessory) being more to the front than the rest. The nerve passes out of the cranial cavity through the condyloid foramen of the occipital bone, outside of which it forms a ganglion, and is in close relation with the sympathetic trunk. After leaving the sympathetic (*see* fig. 237), it divides into two branches, of which the anterior contains the glossopharyngeal, the posterior the nerves which are distributed to the heart, lungs, and other viscera. The vagus itself and its cardiac branch run along side of and in the same direction with the lower of the three petrohyoid muscles, as far as the extremity of the posterior horn of the hyoid bone, into which the muscle is inserted. During this part of its course it is accompanied by the laryngeal nerve, which leaves it just before it reaches the insertion of the muscle. At about the same point it crosses the apex of the lung, passing behind the pulmonary artery, and gives off pulmonary branches which accompany that vessel. Having crossed the lung, the nerve finds its way directly to the sinus venosus, but is so surrounded with grey-looking connective tissue, that in small frogs it is difficult to trace it. As it enters the heart it is closely applied to the superior vena cava and to the wall of the sinus.

74. *Method.*—A frog having been slightly curarized or rendered motionless by section of the medulla, is fixed in the prone position. The sternum is then divided in the middle line, and the two halves of the wall of the chest drawn to either side, so as to expose the pericardium and lungs, while a stout glass rod is passed down the œsophagus. The following objects (*see* fig. 237) are then seen :—1. The two aortæ, parting from each other in the middle line, ascend outwards and upwards close to the cartilaginous tips of the posterior horns of the hyoid bone. 2. From each of these horns muscular fibres are seen to stretch backwards and upwards, towards the occipital region; these are the petrohyoid muscles already mentioned, which originate from the petrous bone, and are inserted into the cartilaginous processes just referred to.

The lower of these nearly parallel bundles of fibres, is the guide to the vagus nerve, which always lies along its lower edge. 3. Following the muscles backwards, they are seen to be crossed by a white nervous cord (the hypoglossal nerve), which ascends upwards and inwards towards the muscles of the tongue. Nearer the middle line, lying somewhat further from the surface, but following the same general direction, another nerve is seen, the glosso-pharyngeal. 4. Crossing upwards to the larynx, over the tip of the inferior horn of the hyoid, the laryngeal nerve is seen. This is the only nerve which is likely to be mistaken for the vagus; it must therefore be traced back for a short distance from the cartilage and divided. It is convenient also to get rid of the hypoglossus.

The vagus, with the muscular slip which accompanies it, can now be readily placed on or between the electrodes. On opening the key, the heart usually stops in diastole, with its cavities full of blood, the arrest not being preceded by any previous slowing. If, however, Helmholtz' arrangement of the induction apparatus is used, and the secondary coil is placed at a sufficient distance, a degree of excitation may be attained which, while it falls short of stopping the heart, is enough to diminish its frequency. With reference to this effect, it is to be noticed that, although it is mainly due to mere lengthening of the diastolic intervals, it is also accompanied with an impairment of the vigour of the ventricular systole; so that if the heart is connected with a manometer (*see* § 63), the manometer rises less during the period of slowing than it did before. Another interesting and important fact is, that the effect does not attain its maximum till several seconds after the commencement of the excitation.

[In this and all other experiments in which it is desired to note the time which elapses between the application of a stimulus and its effect, we use the electrical indicator. It is an arrangement exactly similar to an electrical bell, with the exception that the hammer, instead of striking a bell, writes on the recording cylinder of the kymograph. By a simple mechanical arrangement, the same act which opens the Du Bois' key closes another circuit, of which the electro-magnet of the indicator forms part, and *vice versâ*. This being the case, the instrument makes vertical strokes on the cylinder at the moment that the excitation of the nerve begins and ends.]

75. 2. **Demonstration of the Influence of the Vagus Nerve on the Heart in Mammalia.**—In mammalia, the inhibitory nerves contained in the vagi are in constant action, consequently division of both vagi produces acceleration of the contractions of the heart. In the dog, this effect is much more considerable than in the rabbit, and is attended with an increase of the arterial

pressure which in the latter is absent (*see* fig. 238). On the other hand, electrical excitation of the vagus, whether previously divided or not, retards the contractions of the heart in all animals, and, if the induced current is strong enough, arrests the organ in diastole. (*See* fig. 239 *a*, *b*.)

To show these facts in the rabbit, all that is necessary is to narcotize the animal, to insert a needle in the heart at the upper part of the præcordia (*i.e.*, about an inch to the left of the middle line, at the level of the third cartilage), and to expose the vagi on both sides of the neck. If, now, either nerve is placed between the electrodes, and the key opened, the movement of the needle either stops, becomes irregular, or is merely retarded and diminished in extent, according to the strength of the current. To observe the effect of section, loose ligatures must be placed round both nerves, and the animal then left to itself, while the number of pulsations per fifteen seconds is carefully counted. The two nerves are then divided at once, and the countings repeated. The increase of frequency usually amounts to about twenty per cent. Finally, the peripheral end of one nerve is excited, and the same effects produced as by excitation of the undivided trunk.

In demonstrating the influence of the vagus on the heart in the dog, it is desirable to connect the carotid or crural artery with the kymograph; for the most important effects are those which relate to the changes in the arterial pressure. The preliminary steps of the experiment are those described in § 34. Loose ligatures having been placed round both vagi, and a kymographic observation made, to determine the normal arterial pressure and frequency of the pulse, both nerves are divided simultaneously. The mercurial column at once rises, and the contractions of the heart become so frequent, that the oscillations can no longer be followed by the eye, all that can be distinguished being a vibratile movement of the column. On exciting the peripheral end of either vagus, the same effects are produced as in the rabbit. If the current is sufficiently strong to stop the heart, the mercurial column sinks rapidly, inscribing a parabolic curve on the paper (fig. 239 *b*), the exact form of which depends on the condition of the arterial system; the rate of descent varying inversely as the arterial resistance encountered by the blood in its progress towards the veins. On discontinuing the excitation, the heart begins to beat again, at first at long intervals, subsequently more frequently, the pressure rapidly increasing until (for a few moments) it exceeds that observed before excitation. In man, the trunk of the vagus may in some persons be excited by pressure, and results produced which correspond with those of electrical excitation in animals. Prof. Czermak, of Leipsic, is able, by making pressure at the proper spot on the right side of the neck, to arrest the action of his heart for a few moments.*

76. 3. **Demonstration of the Influence of certain Afferent Nerves, in reflex Relation with the Inhibitory Nerves contained in the Vagus, on the Heart. Bernstein's Experiment.**—The inhibitory heart nerves contained in the vagus are in intimate relation, through the heart centre in the medulla oblongata, with certain afferent fibres contained in the sympathetic system; so that when these fibres are excited, the same effects are produced as if the vagus itself was directly acted upon. This may be shown in the frog as follows:—A frog is secured in the supine position. The pleuroperitonæal cavity is then opened, and the intestines and other viscera are removed, great care being taken not to injure the mesentery or the vessels and nerves which it contains. Nothing now remains excepting the heart resting upon the œsophagus. By carefully dividing the double layer of serous membrane which forms the lateral wall of the cisterna magna on both sides (*see* Chap. II.), the ganglionic chains (fig. 240) are brought into view, along with the *rami communicantes* by which the ganglia are severally connected with the anterior roots of the corresponding spinal nerves. In the thoracic part of the visceral cavity the two aortæ are seen converging downwards, till at the level of the sixth vertebra they meet to form one trunk, from which at its origin the mesenteric artery is given off, to be distributed to the stomach and intestines. If now the two aortæ are raised near their junctions, with the point of the forceps, it is seen that one of the ganglia of the cord sends towards the mesenteric artery a branch which meets with its fellow from the corresponding ganglion of the opposite side, to form a plexus of nerves which surrounds the artery; and that from or through this plexus a nerve or nerves (*nervi mesenterici*) can be traced which follow the vessel towards its distribution. It is in these nerves that the fibres which are in reflex relation with the vagus are contained. To excite them, the best method is to raise the aortæ with the forceps from the bodies of the vertebræ, drawing upwards with them at the same time the two ganglionic cords; then to divide the abdominal aorta and the two cords at the level of the seventh or eighth vertebra, severing at the same time some of the *rami communicantes* on either side; and lastly, to place the two aortæ and the cords which accompany them, on the excitor in such a position that the two ganglia next the junction are in contact with the electrodes. On opening the key, the heart is arrested in diastole, beginning to contract again rhythmically as before, when the excitation is discontinued. To demonstrate that the channels by which stimulation of the mesenteric nerves affects

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* Populäre Vorträge, p. 27.

the heart are the vagus nerves and their centres in the medulla oblongata, the experiment must be thrice repeated; first, after section of both vagi; secondly, after destruction of the medulla oblongata; and thirdly, after destruction of the brain, the medulla remaining intact. In the first and second cases the effect is annulled, in the third it is unaltered.*

77. **Reflex Excitation of the Vagus of the Frog, by Mechanical Means: Goltz' Klopfversuch.**—It is now many years since it was discovered by Goltz that excitation of the ends of the mesenteric nerves by mechanical means produces the same effect as the electrical excitation of their trunks. To show this, a frog is secured on its back, the pleuro-peritonæal cavity opened, and the heart exposed as before. The surface of the intestine is then smartly tapped. After a few moments the heart is arrested in diastole. If the ganglionic cord is then divided on each side opposite the junction of the two aortæ, and the experiment repeated, no effect is produced. Another frog is prepared in the same way, with the exception that both vagi are divided. On repeating the tapping, the result is negative. The same thing happens if, instead of dividing the vagi, the cord is divided immediately below the medulla.

78. **Reflex Excitation of the Vagus in Mammalia.**—The constant action of the inhibitory heart nerves in the higher animals is dependent on the constant action of the centripetal nerves in reflex relation with them. This may be shown as follows:—In a rabbit, the trachea is connected with the apparatus for artificial respiration, and the vagi are exposed in the neck. Thereupon the spinal cord is divided immediately below the medulla oblongata. On the cessation of breathing, artificial respiration is commenced. The cervical sympathetics are then divided, and a needle is inserted in the heart. A succession of observations of the frequency of the heart's action is then made, and both vagi are divided. No acceleration of the pulse rate occurs.

The purpose of the experiment is to show that when the afferent sympathetic nerves which are known to be in reflex relation with the vagus heart nerves are severed, the same effect is produced on the vagus as if it were itself divided. There is no way of accomplishing this directly, without such interference with other nerves as would affect the heart, and thereby render the result ambiguous. The most complete method would be to remove the whole ganglionic cord on both sides. Without reference to the extreme difficulty of such an operation, it is clear that it would involve the accelerator nerves (*see* § 80), and thereby perhaps produce an effect the opposite of that which we intended—a slowing instead of an acceleration of the pulse. So also, when the spinal cord is divided immediately below the medulla oblongata, the effect is modified not only by the destruction of the accelerator nerves, but by the general paralysis of the vasomotor system. Consequently no answer to the question is to be obtained by direct observation of the changes which are produced by any such operation in the rate of pulsation of the heart, so that the end we have in view can only be accomplished indirectly. We already know that both vagi are in constant action, *i.e.*, that the heart is constantly under their inhibitory control; and that when this control is removed by dividing them, the frequency of the pulse increases. It is obvious that this effect can only be witnessed so long as the control is in actual exercise; in other words, that if the vagi are not acting, it would make no difference as regards the heart whether they are divided or not. The consideration of this fact suggests the method which is employed in the experiment above described, which shows that in an animal in which the spinal cord has been divided below the medulla, the rate of the pulse is the same before and after section of the vagi.

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* "Untersuchungen über den Mechanismus des regulatorischen Herznervensystems." Archiv. f. Anat. u. Physiol., 1864, p. 614.

Bernstein has further shown that the same thing happens after destruction of the whole ganglionic cord, or of the cervical part, provided that the spinal cord is at the same time severed at the seventh vertebra. In the dog, section of the cord generally diminishes the frequency of the pulse. There is no such effect in the rabbit. The difference can only be explained by supposing that in the former the activity of the accelerator nerves is less, as compared with that of the nerves in reflex relation with the vagus, than in the latter. In the frog, section of the sympathetic at the level of the junction of the aortæ has no direct effect on the frequency of the pulse, for the same reason, viz., that in this animal the heart-beat is not quickened by section of the vagi.

The influence of reflex excitation of the vagus through the fifth nerve may be easily shown in the rabbit by causing the animal to smell ammonia. The effect is immediate. According to the strength of the ammonia, the heart is arrested in diastole, or the diastolic intervals are lengthened. The inhalation of chloroform, which is so apt to be fatal to rabbits, stops the heart in the same way. When sudden death occurs in man by a blow on the epigastrium, or by drinking a large quantity of cold water, the heart is arrested in diastole by the agency of the same nerves as in Goltz's experiment.

79. Demonstration of the Influence of Increase or Diminution of the Arterial Pressure on the Frequency of the Contractions of the Heart.—The pulse is retarded by *increase*, accelerated by *diminution* of arterial pressure. That these effects are mainly dependent on the inhibitory heart nerves, can be shown in the rabbit as follows:—Ligatures having been passed round the vagus nerve on each side, and a needle inserted in the heart, the fingers of the right hand are placed under the animal's back, while the thumb is firmly pressed upon the aorta, the beats of the needle having been previously counted. On making pressure, the frequency of the contractions of the heart is diminished, and this effect continues so long as the pressure lasts.

Both vagi are now divided and the experiment repeated. The frequency of the pulse is still slightly diminished, but the degree of diminution is not to be compared with the previous effect. This experiment can be made with greater exactitude by applying the pressure to the aorta directly, at the same time connecting the carotid artery with Fick's kymograph. To accomplish the first of these objects, the abdominal cavity is opened in a chloralized rabbit in exactly the same way as for excitation of the left splanchnic nerve. It is then easy to place the thumb directly on the aorta as it passes between the crura of the diaphragm. Tracings are thus obtained which show that, during obstruction of the aorta, the arterial pressure is doubled or even trebled, and the pulse rate much diminished, the *status quo* being re-established when the thumb is removed from the aorta. After division of the vagi, the effect as regards pressure is of course as marked as before, but there is scarcely any slowing of the pulse.

The fact that the effect of aortic obstruction in diminishing the frequency of the pulse is so markedly weakened by section of both vagi, shows that these nerves bear a large part in its production, and therefore that the relation between cause and consequence is in this case not dependent on the lengthening of the systole by resistance, as supposed by Marey. The question, however, remains, whether the mechanical explanation may not be accepted as regards the remainder of effect which is observed after the vagi are divided. There are two reasons why this is not possible. One is, that here, as in other cases when the pulse rate is retarded, the retardation does not signify that the systole is lengthened, but that the diastolic intervals are more protracted. The other reason is, that even after section of the vagi, the retardation of pulse produced by increased arterial pressure is *postponed*, whereas if it were merely mechanical it would certainly be immediate. We must therefore turn to the nervous system for its explanation—either to some influence exercised on the heart by means of accelerator nerves, which after section of the vagi are the only channel by which the heart is in communication with the cerebro-spinal centres; or to excitation of the inhibitory nerves in the heart itself. Considering that in the frog the same effects are produced by exciting the ganglion of the vagus in the cut-out heart as by exciting the vagus itself, and that we have no reason to believe that increased pressure produces any paralyzing influence on the accelerators, we need have little hesitation in concluding that the effect of increased blood-pressure in retarding the heart's rhythm is exercised entirely through the inhibitory heart-nerves; and that it is due principally to the increased supply of blood to the intra-cranial vagus centre, *i.e.*, to the medulla oblongata, but partly also to the influence of the increased endocardial pressure on the vagus ends in the heart itself.

80. Demonstration of the Functions of the Accelerator Nerves.—It has been already seen that when, after severance of the spinal cord just below the medulla oblongata, the organ is excited electrically below the section, two effects are produced: the arterial pressure, reduced by the section, is enormously increased, and the heart beats much more frequently. Bezold thought that both of these effects were due to the direct action of the spinal cord on the heart. Ludwig and Thiry showed that, as regards arterial pressure, this was a mistake. They also showed that the acceleration of the pulse was in part a secondary effect of the increased resistance to the flow of blood; for they found that even after the complete severance of all nervous communication between the heart and the spinal cord, the pulse became markedly more frequent on excitation of the cord. Hence Ludwig was led to doubt whether, after all, the central nervous system exercised any direct accelerative influence on the heart. We now know that while v. Bezold was wrong in believing that the spinal nerves have any power of augmenting the energy of the heart's contractions or of causing it to do more work in a given time, there are certain nerves by which the *distribution* of its efforts in time may be modified in the direction of greater frequency. By the following experiment it can be shown that the acceleration of pulse which is produced by electrical excitation of the severed spinal cord is independent of increase of arterial pressure.

In a curarized rabbit in which respiration is maintained artificially, the spinal cord is severed from the medulla, and the vagi, sympathetics, and depressors are divided. The arterial pressure of course sinks to about an inch of mercury, and the pulse becomes slower. The cord is then excited electrically. The pressure rises at once to four or five inches, the rate of the heart's contractions also increasing, but not in proportion to the rise of pressure. As soon as the effects of stimulation have subsided and the circulation has had time to resume its former condition, both splanchnics are divided, in consequence of which the pressure again sinks a few millimeters. The key is opened; again we have acceleration of the pulse, but this time, the *nervi pressores* having been divided, the excitation produces hardly any effect on the arterial tension. The results of one of Ludwig's experiments are as follows:—After section of the depressors, vagi, and sympathetics, arterial pressure 60 millimeters, pulsations in 15 seconds, 52; after section of cord, arterial pressure 20 millimeters, pulsations 45; during excitation of cord, arterial pressure 80 millimeters, pulsations 61; after section of splanchnics, arterial pressure 10 millimeters, pulsations 27; during excitation of medulla, arterial pressure 12 millimeters, pulsations 42.

81. Proof that the Inferior Cervical Ganglion is the Channel by which the Direct Influence of the Spinal Cord on the Heart is exercised.—Before proceeding to describe the experiments by which this is shown, it will be necessary to give an account of the anatomical relations of the lowest cervical ganglion in the rabbit and dog. It is obvious, from what we know of the anatomy of the cardiac nerves as well in man as in the lower animals, that, with the exclusion of the vagus, the only channels by which the spinal cord can influence the heart directly are the *rami communicantes,* by which it is united with the ganglia. By experiment we learn that the communicating filaments by which the accelerating influence of the cerebro-spinal centres is transmitted, are those which enter the inferior cervical ganglion.

In the rabbit, the trunk of the cervical sympathetic ends at the root of the neck, in the inferior ganglion. This ganglion lies deeply on the surface of the muscles which cover the spinal column *(longus colli),* and consequently to the inner side of the tendinous origins of the *scalenus anticus* from the tranverse processes. It has the œsophagus on its inner side, the vertebral artery on its outer, and lies behind the carotid artery and internal jugular vein. The following are the best guides to its discovery:—Superficially, the junction of the external jugular vein and subclavian vein to form the *vena innominata,* in the angle between which vessels the phrenic nerve appears lying on the scalenus anticus; more deeply, the origins of the scalenus anticus, from the two last cervical transverse processes; and particularly the vertebral artery where it passes to the inside of these insertions, to enter the *foramen transversarium* of the sixth cervical vertebra. The upper end of the ganglion is to be found close to the artery on its inner side. The ganglion receives from above, in addition to the sympathetic trunk, communicating branches from the brachial plexus and from the vagus, and a branch (the so-called *radix brevis*) which accompanies the vertebral artery. Downwards, the ganglion sends (besides those leading to the first thoracic ganglion) branches which go towards the heart. One of the most internal of these is the continuation of the depressor nerve, to be hereafter mentioned, which rather passes by the ganglion than springs out of it, and loses itself in the plexus of nerves between the aorta and pulmonary artery. The communication between the lower cervical and the first thoracic ganglion takes place by two nerves, one of which passes in front of, the other behind, the subclavian artery, before that artery gives off the vertebral. The accelerator fibres enter the ganglion by the vertebral nerve, and thence find their way to the heart through the cardiac plexus already mentioned. (*See* explanation of fig. 241.)

In the dog, the arrangement of the accelerator nerves is somewhat different. In this animal, as in the rabbit, the lower cervical ganglion lies on the *longus colli* immediately to the inner side of the vertebral artery, and above the subclavian. It is connected with the first thoracic ganglion by two twigs, one of which passes behind the subclavian and vertebral arteries, the other in front of them. Of its cardiac branches, of which three have been distinguished by Cyon, the most important accompanies the recurrent nerve until that nerve bends upwards to its distribution, and then follows the subclavian or innominate artery to gain the cardiac plexus. From above, the ganglion receives, first, the combined trunk of the vagus and sympathetic, which here separate from each other, the former continuing its course into the thorax; and secondly, two branches corresponding to those described in the rabbit. The accelerator fibres are very variously distributed among these several branches, sometimes finding their way to the heart from the inferior cervical ganglion along the vagus, or the recurrent, but most frequently by the cardiac branch above described. For further details, see the explanation of fig. 242.

Before entering on any experimental inquiry relating to the accelerator nerves, it is absolutely necessary to make several dissections. The mode of experiment is as follows:—In a curarized rabbit in which artificial respiration is maintained in the usual way, an incision is made in the middle line extending from the upper third of the sternum to the upper end of the trachea. The external jugular vein of one side is then brought into view, tied in two places, and divided between the ligatures. The sterno-mastoid muscle is also divided between ligatures: a strong, threaded aneurism needle is thrust under the sterno-clavicular ligament and the upper fibres of the pectoral muscles; these, with the ligament, are divided between ligatures, and the cut ends drawn aside. By this proceeding, the carotid artery, the internal jugular vein, and the subclavian vein, are brought into view. These veins and the *vena anonyma* are tied and divided in the manner already indicated, and any other vessels which come in the way are secured. A simpler and more rapid mode of performing the operation is the following:—The superficial parts having been exposed by two lines of incision, one of which is in the middle line, while the other extends from it on either side in the direction of the sterno-clavicular ligament, and the jugular vein having been divided between ligatures, the next step is to find the pneumogastric nerve at the upper part of the wound, and free it from the surrounding tissues. This done, a blunt aneurism needle is threaded and passed carefully, with its convexity backwards, along the course of the nerve, between it and the carotid artery. Its point is then made to penetrate the sheath and fascia immediately above the long, cord-like, sterno-clavicular ligament. The thread is then severed, and the ends having been drawn out to a sufficient length, the two ligatures are tightened, the one inside and the other outside of the aneurism needle, after which the whole of the tissues which are tied off between the ligatures, including the great veins, may be raised on the needle and divided. The needle, which has been carefully kept in its place, is now again threaded, and its point pushed downwards under the edge of the pectoral muscles, as far as the upper surface of the first rib. The point is then pushed outwards and forwards through the muscles, the thread is again severed, and the muscles are divided between the two ligatures in the manner already described. By this proceeding a deep hollow (*see* fig. 243) is exposed, in which, among other important parts, the *ganglion inferius* lies, covered by a layer of fascia. This hollow is bounded below by the crescentic upper border of the first rib, behind and to the outside by the *scalenus anticus,* and to the inside by the trachea and (on the right side) by the œsophagus. In the depth of the hollow, to the outside, lies the subclavian artery on its way to cross outwards over the first rib: the vertebral artery springs from it just as it is about to leave the hollow space. This vessel is the guide to the ganglion which lies on its inner side concealed in a good deal of cellular tissue. To find it, the most certain method is to seek for the trunk of the sympathetic in the upper part of the space where it lies concealed behind the carotid artery, and then to trace it down to the ganglion. All this having been accomplished without bleeding, there is no difficulty in passing a ligature round the ganglion, so that at any desired moment it may be extirpated. The same operation is then performed on the opposite side of the body. Both ganglia having been thus prepared with as little loss of time as possible, the sympathetic and vagus are divided (so as completely to sever the nervous connection between the heart and the central nervous system), and one of the carotids is connected with the kymograph.

The medulla oblongata is then divided, and comparative observations are made, in the manner already directed, as to the effect of excitation of the peripheral end of the spinal cord on the arterial pressure, and on the frequency of the pulse before and after extirpation of both ganglia. In the one case, the rise of pressure is attended with acceleration; in the other, the frequency of the contractions of the heart remains unaltered. This result proves, first, that the accelerative influence of the cord on the heart is conveyed by nerves which pass through the ganglia; and secondly, that these nerves are not in constant action. Although the cord when excited acts throughout by means of them, their destruction produces no effect on the heart when the cord is quiescent.—To complete the proof that the nerves which pass to the heart from the sympathetic trunk, and particularly those which spring from the ganglion, are concerned in shortening the diastolic intervals, direct observations are necessary. Such observations were first made by the brothers Cyon, who found that both in the dog and rabbit most of the accelerator fibres reach the ganglion by the nerve which accompanies the vertebral artery. In both animals, but especially in the dog, as has been already stated, the path followed by these fibres from the ganglion to the heart varies considerably in different individuals. The experiments by which these facts have been established are among the most difficult in physiology, and consequently the description of them lies beyond the scope of this work.

From the preceding experiments and observations, we learn that it is the function of the accelerator nerves to shorten the diastolic interval, and thus, indirectly, to render the individual contractions of the heart feeble and less effectual. How they act, and what is their anatomical and physiological relation either to the ganglion cells, or to the vagus of which they are the antagonists, it is not at present possible to explain. As has been already stated, the heart of the frog does not receive any accelerator nerves. From the following experiment, however, it appears that the vagus nerves in that animal contain accelerator fibres. To demonstrate this, the animal must be placed under the influence of nicotin, which alkaloid, as lately shown by Schmiedeberg, possesses the power of paralyzing the terminations of the inhibitory fibres contained in the trunk of the vagus, without affecting the intrinsic inhibitory ganglia of the heart. If in a frog, into which about a thirtieth of a grain of nicotin has been injected, one vagus nerve is excited, the excitation, instead of arresting the heart in diastole, or diminishing its frequency, accelerates its contractions. And if, instead of injecting the solution under the skin, the heart is prepared after Dr. Coats' method, supplied with serum containing nicotin, and connected with the kymograph, and observed before, during and after excitation of the vagus, tracings are obtained which show that the frequency of the heart-beats is increased sixty per cent.; that the acceleration commences about four seconds after the opening of the key, and lasts about a minute and a half after the cessation of the excitation; and that it is due to shortening, or rather annulling, of the diastole, each systole following immediately on the close of the preceding one (*see* fig. 244).

82. Demonstration of the Functions of the Depressor Nerve.—In the rabbit as well as in the cat, a cardiac branch separates itself from the vagus at the level of the thyroid cartilage, high in the neck, and ends in the inferior cervical ganglion. In the rabbit, the nerve commonly originates in two roots, one of which springs from the superior laryngeal, the other from the vagus itself, near the point at which the laryngeal leaves it; but very often it is derived exclusively from the superior laryngeal. In its course towards the inferior cervical ganglion, it is close to the carotid artery, and still closer to the sympathetic trunk, from which it is distinguished by its smaller size and whiter aspect. From the ganglion the fibres of the depressor are continued downwards, forming the two most internal of the filaments which in the rabbit pass between it and the heart. They can be traced to the connective tissue between the origin of the aorta and pulmonary artery. The depressor contains centripetal fibres, the function of which is to diminish the activity of the vasomotor centre, and thereby diminish the arterial pressure.

A rabbit is chloralized; one carotid is connected with the kymograph, and the vagus of the same side divided opposite the thyroid cartilage. The depressor is isolated, and a loop of thread passed round it. An observation is then taken of the arterial pressure and pulse rate, after which the depressor is divided. There is no alteration either in the height of the mercurial column, or in the number of pulsations per ten seconds. On exciting the peripheral end, there is still no effect; but on exciting the central end, the pressure sinks to about two-thirds of its previous height, and the pulse often becomes slower. On discontinuing the excitation, the *status quo* is gradually restored.

The results of such an experiment are shown in the tracing (fig. 245). It is seen that the excitation produces no change whatever either in the character or frequency of the pulsations, the only effect produced being diminution of pressure. In other instances there is perceptible slowing, but the variations of the two effects are never parallel. In the observation recorded in the tracing, the vagus of the side opposite to that on which the depressor was excited, was left intact; consequently the heart was still partly under the control of the intracranial inhibitory centre. Notwithstanding this, the slowing was not appreciable. When it does occur, it must be attributed, without doubt, to reflex excitation of the inhibitory heart centre, the effect of which is conveyed to the heart by the undivided vagus.

The diminution of the arterial pressure cannot be referred to any direct influence exercised by excitation of the depressor on the heart, but to diminution of the resistance in the arterial system; *i.e.*, to relaxation of the minute arteries. This may be shown in the same animal which is used for the preceding experiment, if the left splanchnic is divided (*see* § 56) and the depressor excited as before. The mercurial column, which has already fallen, say, to two-thirds of its former height, is further depressed during excitation; but the amount of sinking is much less than it would have been if the splanchnic had not been divided.

The same conclusion is confirmed by two other observations, viz.: (1) that if the aorta is obstructed so as to raise the arterial pressure and conceal any changes in the state of contraction of the abdominal vessels, the effect of the excitation of the depressor is imperceptible; and (2) that if the abdominal organs are exposed and inspected during excitation of the depressor, they are seen, according to Cyon, to become congested. The effect is most perceptible in the kidneys, which (if care is taken to avoid the previous occurrence of congestion from exposure or other conditions) change colour from pale to red, and back again, as the induced current is closed or opened.

SUPPLEMENT.

Absorption by the Veins and Lymphatics.

Under this head, certain experiments will be referred to relating to the mode in which soluble and insoluble substances find their way into the vascular system from the tissues. This kind of absorption may be termed, in order to distinguish it from that which takes place at the cutaneous and mucous surfaces, *internal absorption*. The other kind will be dealt with in succeeding Chapters.

It is obvious, so far as relates to the blood-vessels, that, considering that the whole vascular system, with the exception of that of the spleen, the medulla of bone, and some other smaller tissues, is lined with a continuous membrane, no substance can enter them excepting in a state of solution, and consequently that the process of venous absorption is one of filtration or diffusion; and that, of these two, the former is excluded by the fact that the pressure inside of the vascular system is everywhere greater than the pressure outside. As regards the lymphatic system, on the other hand, the anatomical facts described in Chap. VIII. will show that there is no obstacle to the entry of solid substances, provided that they are in a state of extremely fine division; so that we are led to infer that, whereas it is the function of the blood-vessels to absorb substances which are soluble and diffusible, those which are incapable of diffusion are taken up by the lymphatics.

From experiments we learn, not merely that this inference is correct, but that the process of absorption from the tissues by the veins is, like the analogous process of secretion (Chap. XXXVI.), dependent on the nervous system.

83. **Proof that Solid Matters in a State of extremely fine Division are absorbed from the Tissues by the Lymphatics.** —In Chapter VIII. it has been shown that (without reference to the origin of the lacteals from the mucous membrane of the intestine, or to the stomata, by which the lymphatic system communicates with the serous cavities) the absorbent system originates from those forms of interstitial tissue which for the present we designate lymphatic, the characteristic of which is that they consist of ground substance, riddled in all directions by cavities containing protoplasm masses, *i.e.*, cells, these cavities being in communication with each other, as well as with the lymphatic capillaries, by a net-work of channels (lymphatic canaliculi or *Saftkanälchen*). The distribution in the body of interstitial tissue having these characters has not yet been sufficiently investigated; for it is only during the last year or two that its anatomical relations have been more or less completely made out. We already know, however, that it is to be found almost everywhere, particularly in the *tunica adventitia* of blood-vessels, underneath the endothelial lining of serous cavities, and of the vascular system, and on the surface and in the inter-fascicular splits of tendons and aponeuroses; and that wherever it occurs, it is in anatomical relation with lymphatic capillaries. The proof that the absorption of solid matters in fine division takes place mechanically, has already been given in Chapter VIII., where it is shown that the lymphatics leading from the peritonæum can be filled with Prussian blue or other colouring matters in suspension, by injecting the liquids charged with them into the peritonæal cavity; and that if the mechanical conditions are favourable, the injection takes place in the same manner in the dead body as in the living. It has also been shown in the same Chapter, that in order to obtain good anatomical preparations of lymphatic capillaries, the best method is that there described as the method of puncture; the reason being that, wherever these vessels are abundant, they are in open communication with the canaliculi, and, consequently, that it is impossible to introduce the point of a syringe into the tissue between them without penetrating many of these cavities. This may be instructively shown as follows.

84. **Method of showing the Mode of Entry of Coloured Liquids into the Lymphatic Vessels.**—The best tissue for the purpose is the mucous membrane of the larynx and trachea: those of an ox or sheep may be used. An ordinary subcutaneous syringe, with as fine a point as possible, is charged with solution

of alkanet in spirits of turpentine. The point is then inserted horizontally into the mucous membrane, at some part where it rests upon cartilage. A drop of the liquid is then pushed out into the tissue as slowly as possible. If the operation is successful, it at once fills the lymphatic net-work, the character of the result varying according as the point of the syringe has entered the submucosa or has not penetrated beyond the mucosa. That the liquid progresses along the vessels by capillarity is learnt by observing that the injection continues to spread long after all pressure from the syringe has ceased. The alkanet solution is employed in this and similar experiments, because it is quite incapable of passing through organic membranes, is immiscible with water, and enters capillary channels with extraordinary facility.

The further progress of liquids along the lymphatics towards the venous system is due partly to capillarity, partly to the fact that the lymphatics pass through spaces in which the pressure is less than that in which their capillaries originate, and partly to the variations of pressure due to muscular action, to which they are subjected. That in certain parts of the body the lymphatic trunks are subjected to a less pressure than their absorbing orifices, does not need special experimental proof. Thus, for example, it is certain that the lymphatics of the peritonæum enter the thorax, *i.e.*, pass from a cavity where the pressure is usually greater, to another where it is much less than that of the atmosphere. The influence of muscular movements admits of being demonstrated by the following experiment, which at the same time affords a striking confirmation of the evidence already given as to the mechanical nature of lymphatic absorption.

In a large dog, which has been just killed by opening one carotid, the skin, costal cartilages, and muscles of the flank are severed by a transverse incision, which extends from the ensiform cartilage as far as the middle line on either side. The wall of the abdomen is then split vertically in the linea alba, and the diaphragm cut away from the ribs. The bladder having been squeezed empty, two ligatures are tightened round the rectum, which is divided between them. Ligatures must now be placed round the cardia, the hepatic vessels and duct, and the mesentery, so as to remove the stomach and intestines *en masse* without bleeding. This having been accomplished, the vena cava is tied above and below the liver and that organ removed, after which the body is bisected by sawing through the eighth vertebra and completing the division of the soft parts. Finally, a glass cannula, fitted with a flexible tube guarded by a clip, is inserted in the thoracic duct and secured with a ligature.

If now the spinal column is fixed near the edge of the table, and the lower limbs alternately flexed and extended by an assistant, the lymph flows freely and may be received in a test tube. If the passive movement is discontinued and then resumed from time to time, the quantity of lymph collected is very considerable, so that it is easy to fill several test tubes; but none is discharged during the intervals of cessation. The lymph which is collected at first, resembles ordinary lymph both in its microscopical characters and in its composition. It is obvious that it is the liquid which at the moment of death occupied the canaliculi of the tissues from which it is gathered. The course taken by the lymph stream can be further demonstrated in the same preparation, by introducing solution of alkanet, by puncture, into the intertendinous splits of the lower part of the *fascia lata*. If a sufficiently fine syringe is used, it is easy to produce in this way a satisfactory injection, first, of the lymphatic capillaries contained in the splits themselves, and secondly (if the passive movements are continued), of the rich net-work of lymphatics which exists in the "cellular membrane" which covers the aponeurosis on its cutaneous aspect.* Soon the discharge from the thoracic duct is reddened by the alkanet. It has been shown by Ludwig that in the extremities, the tendons and aponeuroses are the special seat of the net-works of capillaries by which the lymphatics commence, and that they have here an arrangement similar to that observed in the central tendon of the diaphragm. The experiment proves that even passive movements of the limbs, by alternately tightening and relaxing these structures, press forwards the lymph stream. The influence of active movements must be much greater.

85. **Internal Absorption by the Veins.**—The proposition stated at the beginning of the section, that substances in solution enter the capillaries from the tissues by a process of absorption, which is under the immediate control of the nervous system, may be strikingly illustrated as follows:—

Two frogs having been slightly curarized are prepared thus: The heart having been exposed *lege artis*, a small opening is made in the skin in the occipital region. In one of the frogs, the brain and spinal cord are completely destroyed by passing a needle upwards and downwards from the occipital region, and then both are hung vertically on a board, side by side, looking in the same direction. A small funnel, the stem of which is drawn out into a narrow beak, is now passed from the incision downwards under the skin of each animal, till its end reaches the dorsal lymphatic sac. This done, the bulbus aortæ is divided in both animals, and the results are observed. In the frog deprived of its central nervous system, only a few drops of blood escape,—the quantity, that is to say, previously contained in the heart and in the beginning of the arterial system. In the other, the bleeding is not only more abundant, but continues for several minutes after the section. As soon as bleeding has ceased, a quantity of saline solution (say, 5 to 10 centimeters) is injected into the lymphatic sac of each animal until it is distended, and the exact quantity used carefully noted. In the frog in which the central nervous system is intact, the discharge of blood from the opening in the bulb begins again, and goes on increasing; while the liquid, which at first is nearly pure blood, becomes more and more diluted with serum. The discharge of sanguineous liquid goes on for one or two hours; and if, during the progress of the experiment, the vasomotor centre is stimulated reflexly by exciting a sensory nerve or the surface of the skin, it is seen that the rate of flow is at once augmented, but becomes less after the cessation of the excitation than it was before. This last fact is thought by Goltz,* the author of this experiment, to indicate that when a sensory nerve is excited, venous absorption is increased. It may perhaps be attributable rather to the contraction of the vessels which is determined by the excitation. To render the observation of the result as accurate as possible, the quantity discharged should be measured. The quantity found in the test-glass in which the mixture of blood and serum is collected should, together with the residue remaining in the lymph sac, be equal to the quantity originally injected.—In the other frog there is no discharge. The heart remains flaccid although contracting regularly, and the skin dry from the arrest of the secretion of the cutaneous glands. In this experiment it may be supposed, either that the liquid contained in the lymph sac passes into the circulation directly, or that it first diffuses out into the surrounding tissue, and is then absorbed by the veins. The first supposition is negatived by the observation that the contractions of the lymph hearts have ceased in both frogs, and that consequently the mechanism by which alone the liquid could be directly transferred to the venous system is wanting. We are therefore compelled to admit that it enters the blood stream by the only other channel open to it; and the conditions of the experiment prove that it does so under the direct influence of the nervous system.

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* Coloured drawings of the injections so obtained will be found in Ludwig and Schweigger-Seidel's beautiful monograph on the lymphatics of tendons and aponeuroses.

The precise nature of the agency by which the living elements which surround the blood-vessels determine the diffusion of liquid into the blood in opposition to pressure, cannot at present be stated. In the instance before us, two sets of effects may be distinguished as referable to one cause, *i.e.*, destruction of the central nervous system,—those due to paralytic relaxation of the blood-vessels, and those which are attributable to absence of absorption. In how far those of the second kind are the immediate result of the others, may perhaps be open to question. They do not, however, afford any explanation of them, for there is no reason why a relaxed vessel should not absorb quite as much as a contracted one; the fact of relaxation affords no explanation whatever of the absence of absorption. Both are manifestations of properties enjoyed by the living elements only so long as they are in communication with cerebrospinal nervous centres.

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* "Ueber den Einfluss der Nervencentren auf die Aufsangung." Pflügers Archiv. B. v., p. 53.

CHAPTER XVII.

RESPIRATION.

SECTION I.—PRELIMINARY STUDY OF THE EXTERNAL MOVEMENTS OF RESPIRATION.

86. Respiratory Movements of the Frog.—To observe the respiratory movements in the frog, the animal must be fixed on its back. It is seen that that part of the floor of the pharyngeal cavity which corresponds to the submaxillary space, *i.e.*, to the space which lies between the episternal cartilage, and the two branches of the lower jaw bone, alternately rises and falls at intervals of about one or two seconds. On more attentive examination, it is found that these movements are due to the alternate retraction and advance of the body of the hyoid bone, the general form of which can be readily distinguished under the skin. To study their nature, the skin must be divided in the middle line from the mouth to the sternum, and detached from the subjacent muscles as far outwards on either side as the jaw. In this way a view is obtained of all the muscles attached to the hyoid bone, without interfering with the mechanism of respiration (*see* fig. 246). By its long and slender anterior horn, the hyoid bone is connected with the skull (*i.e.*, with the cartilaginous part of the petrous bone) in such a manner that, although the two cartilages are not united by a joint, the hyoid works on the petrous bones as if it were hinged to them. This being borne in mind, it is easy to understand the action of the muscles which are attached to it. Those which come from the sternum and bones connected with it, in drawing the hyoid backwards, cause it, at the same time, to descend in such a way as to increase the space between its upper surface and the roof of the mouth and pharynx, and to extend that part of the submaxillary space which intervenes between the arch of the hyoid and that of the lower jaw. On the other hand, those muscles which stretch from the chin (the genio-hyoid), and from the petrous bones (the petrohyoid muscles) to the body of the bone, combine in drawing it upwards and forwards, to such a degree, indeed, that when the latter are in action, the submaxillary space becomes concave. All this can be readily seen in the living animal; for although the muscles above-mentioned are covered by the submaxillary or mylohyoid muscles, this muscular membrane is so thin that they can be easily perceived through it.

To investigate the part taken by these movements in the mechanism of respiration, it is necessary to ascertain in what relation they stand to the influx and efflux of air. This is accomplished by inserting a suitable glass cannula into one of the nostrils and connecting it with the tympanum, shown in fig. 231. In this way the curve is obtained, which is copied in fig. 246 *bis*. By watching, at the same time, the motions of the hyoid bone and of the lever, it is easy to satisfy one's-self that the retraction of the former towards the sternum corresponds with the depression of the latter, and with the entrance of air into the pharyngeal cavity. It is further seen that the motions are by no means uniform, and that in connection with this want of uniformity they present certain peculiarities which, from their intimate connection with the mechanism by which air is introduced into and expelled from the lungs, require careful attention. The tracing enables us to divide the respiratory acts into two kinds, viz., smaller alternative movements (*a a a*), which occur at pretty regular short intervals, and larger movements (*b b b*), which differ from the others in this respect, that the less energetic expiratory act by which the movement begins, terminates in a sudden expulsion of air, indicated by a more rapid rise of the lever, and determined by a more vigorous contraction of those muscles which connect the body of the hyoid bone with the skull. This sudden elevation of the floor of the pharynx is the act by which the frog injects air into its lungs. The student must now fix his attention on the nostrils, when he will see that whereas during the small movements (*a a*) those organs are motionless, the sudden expulsions (*b b*) are accompanied by contraction of the little constrictor muscles of the nares, and, consequently, that the latter differ from the former not merely in their greater vigour, but in their being executed with the nostrils more or less closed, so that the air, instead of passing freely out, is injected through the glottis into the lungs. To prove this, watch the expiratory muscles of the flanks (the external oblique particularly). At the first moment, it will perhaps appear as if the sudden contraction of these muscles were coincident with the closure of the nares, but it is soon seen that the former movement follows the latter at an interval of time, which, although very short, is not difficult to appreciate even without instruments. This may be demonstrated graphically by puncturing the anterior wall of the visceral cavity, and introducing through the puncture a cannulá in such a way that it communicates with the cavity of one lung. The cannula being connected with a tympanum, a tracing is obtained, which shows that the period during which the air is contained in the lungs is extremely short, that the entry of air into the lungs coincides with the closure of the nares, and is determined by the approximation of the body of the hyoid bone to the roof of the pharynx, and that the expulsion of air from the lungs by the contraction of the flanks occurs while the hyoid is still drawn upwards, so that the two muscular movements form part of the same act.

87. **External Respiratory Movements of Man and Mammalia.**—The alternate emptying and filling of the air cells of the lungs, which is the final cause of respiration, is effected by the alternate enlargement and contraction of the chest. If the whole of the thorax were occupied by the air cells, these changes of capacity could be measured by the quantity of air entering and leaving the respiratory cavity in each act of breathing. As however, in addition to the lungs, the chest contains various other organs, some of which alter their volume very considerably, according to the degree of expansion of the cavity in which they are contained, there is no constant relation between the enlargement or diminution of the available intra-thoracic air-space and the external enlargement or diminution of the thorax.

There is no practicable method of determining the changes of volume which the chest undergoes in respiration with exactitude. As, however, the imperfect methods we possess differ from most of those employed in physiology, in being quite as applicable to man as to the lower animals, and are sufficiently accurate to yield valuable results in the study of disease, they are well worthy of the attention of the physician, though of comparatively little interest to the physiologist.

88. The external movements of the human chest may be investigated by recording the variations either of its diameters or of its circumference, at different parts, or of both simultaneously. For the graphic measurement of the circumference, an instrument, contrived by Marey, and much improved by Bert, is used. It consists of an air-tight cylinder of brass and india-rubber, of the shape and construction of a common drum, the cylinder being of brass and the membranous ends of india-rubber. The cylinder communicates by a flexible tube with a tympanum, the lever of which records its variations of capacity. To the centre of each of the two terminal membranes a metal disk is attached, which is furnished with a hook, and is thus connected with one of the ends of an inelastic cincture, which encircles the circumference to be measured. As the circumference augments, the membranes are extended, and the capacity of the drum increased, and *vice versâ*. It is obvious that before

the instrument is used it must be graduated. The mode of accomplishing this will be given further on.

89. The graphic measurement of the diameters of the chest is much more simple, inasmuch as it merely involves the translation to the paper of the movement produced by the alternate recession from each other, and approximation to each other, of two points in the chest wall at the opposite extremities of the diameter to be measured ; so that if either of these points be taken as fixed, the recording of the movement of the other point amounts to nothing more than the conversion of one rectilinear movement into another. This is readily accomplished by the contrivance we have already employed for recording the external cardiac movements (see § 60), that is, by the employment of two tympana, the one for receiving the movement to be investigated, the other for inscribing it on the cylinder. The receiving tympanum must be so placed that the distance of its india-rubber membrane from the *fixed* extremity of the diameter to be investigated, is subject to no variation during the period of measurement, and that its ivory button is applied to the movable end in such a way that the diameter, if produced beyond the surface of the chest, would coincide with its axis. All these conditions are completely fulfilled in the instrument I use. It consists of two parallel bars of iron, the opposite ends of which are screwed firmly at right angles into a cross bar, so as to form a rigid frame resembling in shape the Greek letter Π. The diameter to be investigated is placed between the extremities of these bars. One of these carries an ivory knob, similar to that of the cardiograph, the convexity of which looks towards the opposite arm. Its distance may be varied by a screw. The other arm bears the receiving tympanum, the knob of which faces the knob just mentioned, their axes being in the same line.

The mode of application of the instrument, which may be conveniently called a recording stethometer, varies according to the diameter to be measured. The most important diameters are those which connect the 8th rib in the axillary line with the same rib of the opposite side, the *manubrium sterni* with the 3rd dorsal spine, the lower end of the sternum with the 8th dorsal spine, and the ensiform cartilage with the 10th dorsal spine. The mode of application for the first of these diameters is shown in fig. 247. The subject stands or sits, as is most convenient, and the stethometer is hung over his neck by a broad band, the length of which can be regulated by a buckle. The movements recorded are not those which the middle of the 8th rib performs in relation to its sternal and vertebral attachments, but those which the one end of the diameter executes in relation to the other, which is for the moment regarded as a fixed point. In measuring the diameters which lie in the middle plane, it is most convenient to take the vertebral spines as fixed points, although, of course, the results would not be affected by doing otherwise. The records obtained by the stethometer are of value for two purposes, viz., for the appreciation of the *relative and absolute duration* of the respiratory acts, and for the measurement of their *extent*. For the latter purpose, the instrument must be *graduated* every time that it is used. To facilitate this process, I employ a set of five standard wooden measures of length, differing from each other by two millimeters. With these, the graduation is effected in less than five minutes. The recording and receiving tympana having been brought into communication, and the whole system tested as regards the perfection of the joints, and found to be air-tight, one of the wands (the one of which the length is equal to the mean of the five) is placed between the two buttons of the stethometer, which are then approximated until the india-rubber membrane of the tympanum is slightly concave. A horizontal tracing having been drawn on the cylinder, the two next longest and shortest wands are substituted for the first, and the process is repeated in respect of each, and then the next two, until five parallel horizontal lines have been drawn, by comparison with which, the variations of the diameters investigated may be estimated in millimeters from the vertical measurements of the tracings. By this method we learn, for example, that in a healthy muscular young man, aged 22, the diameters above given vary respectively in each respiration as follows: Upper sternal diameter = 146, varies one millimeter; the lower sternal diameter = 203, varies 1·5—1·8 millimeter; the transverse costal diameter = 228, varies 1·7—2·0 millimeters.

As regards the duration and succession of the respiratory acts, the most instructive curves are the costal and lower sternal (*see* fig. 248). It must be carefully borne in mind that they apply strictly to natural respiration. In forced breathing, the thoracic movements acquire a different character. Dr. Arthur Ransome, who has studied the subject with great accuracy, allows me to refer to his measurements. He has found that the variations of the antero-posterior diameters of the upper part of the chest are very extensive, and that the whole thoracic framework participates in them—the ends of the upper ribs moving horizontally forward, *i.e.*, in a plane parallel to the middle plane of the body, from 12 to 30 millimeters; the advance of the third rib is greater, by several millimeters, than that of the fifth. Dr. Ransome considers that this advance cannot be otherwise accounted for than by an actual bending of the ribs.

We shall see afterwards that the difference between natural and forced breathing consists partly in increased constriction of the chest during expiration, partly in increased expansion during inspiration. In the mean time, it is sufficient to note that when the thoracic movements become excessive, the change affects the antero-posterior diameters of the upper part of the chest more than of the lower part, so that the normal relation between the two is reversed.

90. Measurement of the Intra-Thoracic Pressure. — In consequence of the elasticity of the lungs, and of the fact that they are contained in a cavity of which the capacity is much greater than the volume which these organs assume in their unextended condition, and that their external surface is inseparably applied to the inner surface of the cavity, the pressure to which the heart, arteries, veins, and other intra-thoracic organs are subjected, is considerably less than that of the atmosphere. What is required to measure the difference is to connect one pleural cavity with a manometer. This is easily effected in the following manner: —A glass tube of about three millimeters in diameter is sealed at one end, and drawn out to a blunt point. A hole is then cut with a sharp three-cornered file on one side of the tube, close to the sealed end, and the open end temporarily closed with a plug of wax. A rabbit having been secured on the rabbit support, the skin is perforated with a scalpel close to the left edge of the middle of the sternum. This having been done, the point of the tube is easily passed into the right pleura by pushing it in a horizontal direction behind the sternum, with its point against the posterior (*i.e.,* as the animal is placed, the under) surface of the thoracic wall. The wax plug is then removed, and the open end is connected with a water manometer; but while this is being done, great care must be taken to keep the side of the tube on which the orifice is, firmly but gently applied against the chest wall. The quantity of water in the manometer is then increased or diminished until the two columns stand at the same level. If now the tube is twisted round so that its orifice looks towards the cavity of the chest, the distal column sinks, the difference between the heights of the two columns in millimeters being about thirteen times as great as the difference in millimeters of mercury between the atmospheric pressure and that to which the thoracic organs are subjected in the animal under observation. The intra-thoracic pressure may also be measured indirectly immediately after death, by connecting the trachea air-tight with a manometer, and then, after seeing that the two columns stand at the same level, opening both pleural cavities. This time the distal column rises above the proximal. The difference between them, if the same kind of animal is used, will be the same though in the opposite direction. If it is desired to obtain a record of the variations of intra-thoracic pressure during the respiratory acts, it is easily done by bringing the tube into communication with a Marey's tympanum, by means of a somewhat thick walled india-rubber connector. In this way the tracing fig. 249 is obtained.

SECTION II.—STUDY OF THE MODE OF ACTION OF THE MUSCLES OF RESPIRATION.

In man, the entry of air into the chest in tranquil breathing is accomplished exclusively by the diaphragm. In the dog, it is effected partly by the descent of the diaphragm, partly by the widening of the chest. In the rabbit, the respiratory movements resemble in their general character those of man, on which account this animal is preferable to any other for the purposes of study. From the fact just stated, it is obvious that in our examination of the action of the muscles of respiration, we must not confine ourselves to the normal breathing, for if we were to do so, our studies would relate almost exclusively to one muscle. To observe the action of the others, we must direct our attention to the excessive thoracic movements of animals affected more or less with *dyspnœa*, the phenomena of which condition, so far as they relate to the action of muscles, must therefore be entered upon here, although its nature and cause will form the subject of a special section.

The muscular movements by which the chest is expanded, must be studied in their relation to a certain definable position of the thorax, which is called the *position of equilibrium.* It is the position assumed by it at the end of normal expiration. For as no muscle takes part in the normal expiratory act, the whole thoracic muscular apparatus is at that moment in a state of rest, the bones and cartilages assuming that position which results from the balance of the opposed elastic forces, which act upon them from within and from without. Of these elastic forces, the most important is that of the lungs; which organs, being contained in a cavity much larger than they are themselves, to the inner surface of which their external surface is inseparably applied, constantly draw together its walls with a force to be investigated in a future paragraph. Next in importance is the elasticity of the ribs and cartilages, by virtue of which the thoracic wall ever tends to be larger than it is, in opposition to the contractile influence of the lungs. Co-operating with these, we have, thirdly, the "tonus" of the thoracic muscles, different indeed in its nature, but indistinguishable as regards its action. All the muscles by which the chest is enlarged beyond the position of equilibrium are called inspiratory; all those by which it is contracted to a capacity less than that which it possesses when in equilibrium are called expiratory.

91. **Inspiratory Muscles.**—*The Diaphragm.*—To demonstrate the action of the diaphragm, several methods may be used. The most striking is to expose it in an animal made completely insensible by the injection of from twenty to forty minims of a ten per cent. solution of chloral into the crural vein. For this purpose the abdominal cavity must be opened in the *linea alba,* immediately below the ensiform cartilage, and then two incisions must be made, extending from the opening in opposite directions parallel to the edges of the costal cartilages, according to the instructions given in § 56. Another method consists in merely making an opening in the *linea alba,* close to the ensiform cartilage, sufficient to receive the finger, the tip of which must be pressed against the centrum tendineum, when the movements can be appreciated with great exactitude. The plan most used consists in introducing a long and slender needle into the chest through the ensiform cartilage, close to the lower end of the sternum, the direction of which is such, that it grazes the upper surface of the diaphragm, if possible piercing it at one or two points, so as to be in some part of its course on the abdominal side of the membrane. For this experiment, the rabbit must be carefully chloralized, and secured on Czermak's supporter in such a way that the spinal column is immovable. A long silk thread is then passed through the eye of the needle and connected with the little box-wood pulley shown in fig. 250, the movements of which are inscribed by means of the horizontal lever on the blackened cylinder. The tracing so obtained enables us not only to determine to what relative distance the dome of the diaphragm descends in each inspiratory act, but also the *mean* relaxation of the muscle, *i.e.*, the mean height to which it ascends during each expiration. This, as we shall see further on, is much affected by conditions which act on the muscle by its motor nerve.

92. *Intercostal Muscles.*—To demonstrate the action of the intercostal muscles, a rabbit is used which has been deprived both of voluntary motion and of sensibility by the ablation of the cerebral hemispheres as well as of the *corpora striata* and *thalami optici.* This operation is performed as follows:—The animal having been rendered insensible by chloroform, both carotids are tied. It is then secured on the supporter in the prone position. The

calvarium is now exposed by an incision extending from the occiput to the frontal region in the middle line, and the integument drawn aside in either direction. The parietal bones having been first perforated with the trephine, to allow of the introduction of the cutting pliers, the roof of the cranium is rapidly removed so as to expose the hemispheres completely. These organs are then scooped out with the ivory handle of a scalpel, an assistant being at hand with the actual cautery to arrest the bleeding. The animal at once passes into a state resembling deep sleep, breathing regularly, but much more slowly than before the operation. The action of the respiratory muscles of the chest can now be investigated without any misgiving as to the infliction of suffering, by removing the integument and superficial layer of muscles so as to expose the ribs and intercostal spaces.

The first lesson to be learnt relates to normal breathing. It is seen that so long as air enters the chest freely, and the respiratory apparatus is not interfered with, there is no appreciable expansive movement of the ribs, and the intercostal muscles, in so far as they are visible, do not contract. Dyspnœa may now be produced, either by letting air into one or both of the pleural cavities, by diminishing the opening by which the chest communicates with the atmosphere, or by combining the two methods. It is most convenient to begin with puncturing one pleura.

The effect of this operation is to increase the respiratory movements, and to alter their character by bringing the thoracic muscles into action. The upper ribs, particularly the second, third and fourth, which were before motionless, move upwards and outwards in each inspiration, while the external intercostal muscles, and the intercartilaginous parts of the internal intercostals are seen to grow hard in contraction at the same moment.

To produce a higher degree of dyspnœa, the other pleura may be opened; the principal effect observed is that the upward and outward movement of the upper ribs is increased, and that the *scaleni*, which were before inactive, now begin to contract in concert with the external intercostals. These muscles, although from their anatomical arrangement they must act as elevators of the ribs, cannot be shown to be so experimentally; for there is no appreciable diminution of the costal movement when they are divided.

The function of the external intercostals and intercartilaginous muscles having been proved by direct observation in the manner above described, at a very early period in the history of physiology, has never been seriously disputed. This is not however the case as regards the interosseous part of the internal intercostals—that part of those muscles which is covered by the external intercostals. In the rabbit, the interosseous intercostals differ from the intercartilaginous, both in being less oblique and in being somewhat thinner. The experimental evidence as to their function is negative—that is to say, it can be shown that they do not contract with the external muscles, but it cannot be shown that they act antagonistically to them. It admits of demonstration as regards any of the lower ribs, from the fourth to the eighth or ninth, that if all the muscles attached to it from above are removed, excepting the external intercostals and the *levatores costarum breves* (muscles which connect each transverse process with the rib next below it, and can be seen to contract with the external intercostals), the rib still rises outwards in inspiration; but if these muscles are completely severed, no more costal movement is perceptible; nor is there any hardening of the exposed inter-costal muscles at the moment of inspiration. In a word, it must still be admitted that the action of these muscles is as yet undetermined. Most probably they may be regarded as constrictors of the chest—as the agents of forced expiration.

93. Movements of the Nares and Larynx.—In the rabbit, the nostrils dilate with each ordinary inspiration, and contract in expiration; but from their frequency these movements are very difficult to observe. To study them satisfactorily, the student must avail himself of the excessive and infrequent respirations of animals in which both vagi have been divided. It is then seen that the dilatation of the nares is the first act of inspiration. It precedes by a distinct interval the expansion of the chest, and appears even to precede the contraction of the diaphragm. Whether it actually does so is very difficult to determine. The muscles by which this movement is effected are, the *subcutaneus faciei* which springs from the lateral surface of the intermaxillary bone, and from the anterior supraorbital process of the frontal bone, to be inserted into the skin of the nose and forehead, and the *levator nasi*, which springs from the lower edge of the orbit, and is also inserted by a long tendon into the skin, covering the edge of the nose. Of the two, the former is the more superficial.

The respiratory movements of the larynx in the rabbit are scarcely perceptible in perfectly natural breathing; but the slightest interference with the access of air to the chest is sufficient to produce them. The larynx is drawn downwards in inspiration by the muscles connecting it with the sternum, and returns to the position of muscular equilibrium in expiration. One of these muscles, the sterno-thyroid, has also the effect of tilting forwards the thyroid cartilage, so as to bring its lower edge nearer the cricoid.

94. To study the intrinsic respiratory movements of the larynx, the *rima glottidis* must be exposed to observation, by making a suitable opening either above or below. The best view of the movements is obtained by dividing the hyothyroid membrane. The skin having been carefully divided in the middle line, *lege artis*, the membrane must be exposed with the aid of two pairs of forceps. The veins (which are the principal source of difficulty) can then be readily seen, and must be carefully secured above and below by ligatures, between which the membrane may be cut across without risk of hœmorrhage. The head must of course be so supported that a strong light is thrown on the vocal cords. If now the epiglottis is drawn forwards, the motions of the vocal cords, and of the arytænoid cartilages are well seen—the chink becoming wider in inspiration, narrower in expiration. To observe the motions of the arytænoid cartilages, the best way is to excite the recurrent nerves, when it is seen that during excitation the vocal cord of the same side approaches the middle line. If both recurrents are excited, the rima is completely closed, the arytenoid cartilages applying themselves to each other just as they do in the production of a musical note. Considering that the recurrent nerve is distributed to all the muscles, and not merely to those which act as constrictors (*arytænoidei and crico-arytænoidei laterales*), and that the movements produced are of the same nature as those which occur in ordinary expiration, though much more vigorous, we arrive at the inference that in both cases the widening of the glottis is a condition of general muscular relaxation, or, in other words, that all the intrinsic muscles of the larynx are expiratory—their combined effect manifesting itself in approximation of the vocal cords, not because the posterior crico-arytænoid muscles and the other dilating muscles do not act with the rest, but because they are overpowered by them.

SECTION III.—MEASUREMENT OF THE QUANTITY OF AIR RESPIRED IN A GIVEN TIME, AND OF THE VOLUME OF AIR INHALED IN EACH RESPIRATORY ACT.

95. The apparatus for this purpose consists of three parts, viz.: (*a*) a receiver or chamber in which the air to be breathed during the period of observation is contained; (*b*) a face-piece and tube for connecting the receiver with the respiratory cavity of the subject of observation; (*c*) arrangements for supplying fresh air to the receiver, to take the place of the air breathed.

To obtain results which are reliable, the first and most important condition is that the air should be respired without the slightest effort. To ensure this, the receiver must be of such construction that the pressure to which the air contained in it is subjected should be the same as that of the atmosphere. Consequently, it must have the form either of a gasometer, the cylinder of which is accurately counterpoised, or that of a membranous bag, the material of which is so thin that it offers no resistance either in contracting or expanding. The best material for the latter purpose is vulcanized india-rubber; it is, however, difficult to obtain bags of this description which are perfect. Whatever be its form, the receiver must have two openings, one communicating with the face-piece, the other for the reception of air. It must be also so constructed that the moment at which it is full may be easily and accurately observed.

The receiver is brought into communication with the expiratory cavity of the subject of experiment by means of a face-piece or mask of very perfect construction, furnished with two valves, by one of which the air is expelled, while the other, opening inwards, guards the orifice of a tube about an inch in width, which leads from the receiver.

By its second opening, the receiver communicates with a gasometer filled with air, under a pressure somewhat greater than that of the atmosphere. Between the gasometer and the receiver, the tube of communication passes first through a stop-cock of brass, the aperture of which can be regulated very accurately by means of a long handle, and then through an accurately graduated gas meter, specially constructed for the purpose. Each observation lasts ten minutes. The gasometer is kept full of air by means of a pair of bellows, which must be worked by an assistant (in default of other motor) during the whole period; while the quantity of air which is driven through the meter to the recipient is so regulated with the aid of the stop-cock, that the receiver is kept exactly at the same degree of fulness.

The chief mechanical source of inexactitude in this apparatus is to be found in the imperfect closure of the valves, and imperfect fitting of the face-piece. These defects may be obviated by substituting for the face-piece a couple of tubes of ivory, which accurately fit the anterior opening of the nostrils. The wide tube with which these ivory nose-pieces are connected, at once divides into two branches. Of these, one is guarded by a mercurial valve leading outwards, the other by a similar valve leading inwards for inspiration, the arrangement of these valves being the same as that shown in fig. 251 to be immediately described.

96. In making observations of the same nature on the lower animals, it is convenient to use an apparatus which not only admits of accurate measurement of the quantity of air breathed,

but renders it possible to modify its composition by the introduction of definite proportions of other gases or vapours. And inasmuch as in such investigations it is, as a rule, of more importance that the conditions should be accurately known than that they should be identical with those normally existing, the principle of completely avoiding resistance, which was regarded as fundamental in the construction of the apparatus described in the preceding paragraph, must be abandoned; for it is a mechanical impossibility to construct valves which, while they close with perfect accuracy, work without resistance. The apparatus to be now described is so constructed that any gaseous mixture may be kept in it for a length of time without change of composition by diffusion, and the valves act so perfectly that the experimenter is absolutely certain that the whole of the air which leaves the receiver, and no more, is actually used in respiration.

The receiver may be constructed as follows: Two glass cylinders are selected, about eight inches in length, open at both ends, one of which is about half an inch wider than the other; the outer is about three inches in width, the inner (E fig. 251) two and a half inches. Both of them are cemented in the most perfect manner possible, with their axes in the same vertical line into a circular horizontal plate, so that they are separated from each other by a narrow space of the same width everywhere. This space is to be filled with mercury. Through the central part of the plate rise three vertical tubes of glass, of about a quarter of an inch internal diameter. Underneath the plate, which is supported on a tripod, each of these tubes passes downwards for a short distance, and is then bent horizontally at right angles. A third cylinder (D), closed at one end and made of iron carefully protected, constitutes the bell of the gasometer. Its diameter is the mean of the diameters of the two cylinders of glass, so that it descends without touching them into the space containing mercury, by which they are separated from each other. It is suspended by a silk cord pulley and counterpoise. The counterpoise consists of a cup containing shot, and there is a second and similar cup on the top of the cylinder. Of the three tubes which enter the receiver from below, one (A) communicates with the atmosphere (when in use), a second (B) with the respiratory cavity of the animal, the third (C) is usually closed. The india-rubber tubes by which these communications are made, are guarded by the simple contrivances, known as Müller's mercurial valves. Each such valve consists of a rather wide bottle containing a shallow column of mercury, and closed airtight with an india-rubber stopper. Through the stopper pass two tubes, one of which is of such length that its end dips just below the surface of the mercury; the other is much shorter. The valve (A) is so placed that the short tube is in direct communication with the receiver, the long one with the atmosphere; in (C) this arrangement is reversed. To complete the apparatus, all that is required is a T tube and a third valve. The stem of the T tube communicates with the respiratory cavity by means of a cannula secured air-tight in the trachea; the one arm with the receiver, and the other with the valve (B), through which the expired air is discharged into the atmosphere. The quantity of air used by the animal during any given period of observation must be measured in the same way as before.

The objection to which this apparatus or any other of similar construction is liable, lies, as has been already hinted, in the resistance offered by the mercurial valves, which is sufficient to retard the respiratory movements to a sensible degree. As, however, the most important applications of the method are those which relate to the influence of variable conditions on the quantity of air breathed, this fact is of little consequence; for the error arising from it may be entirely eliminated by substituting, as a standard of comparison, the respiration already modified by the resistance, for normal respiration. For the purpose of obtaining such a standard, the animal must be allowed to breathe common air through the apparatus for some time before making any other observation.

SECTION IV.—DETERMINATION OF THE QUANTITY OF CARBONIC ACID GAS DISCHARGED BY AN ANIMAL FROM THE LUNGS AND SKIN IN A GIVEN TIME.

97. There are two leading methods by which this can be accomplished. One of them is that of Regnault and Reiset, which, with important modifications, has been used by Ludwig and his pupils. The animal under observation is contained in an air-tight chamber, which communicates with a second chamber containing oxygen. The chamber communicates with an absorbing apparatus, through which the air passes in a continuous current, so that the expired carbonic acid gas is removed from it as rapidly as it is formed, its place being taken up by exactly the same volume of oxygen, so that the constitution of the air remains unchanged. The quantity of carbonic acid gas absorbed is calculated from the increase of weight of the absorbing apparatus during the period of observation. As improved by Ludwig, the method is the best suited for exact experiments. The apparatus is described in Ludwig's *Arbeiten* for 1869.

The second method, which is much simpler, and sufficiently exact when for comparative investigations as to the influence of various physiological and pathological conditions on the discharge of carbonic acid gas, is that of Pettenkofer. It is applicable either to large animals or small. A short account of Pettenkofer's complete apparatus will now be given, as an aid to the understanding of the application of the same method to the small animals in common use for physiological and pathological investigations. Pettenkofer's apparatus consists of three parts, viz., a chamber in which a man can sit or stand comfortably; a large wet gas meter, which communicates with the chamber by a tube; a double-action air-pump, by which air is continuously drawn through the meter from the chamber; and, lastly, clockwork, by which the pump is worked. The chamber, which is of metal and glass, communicates with the external air during the period of observation by the interstices round the door, which serve for the entrance of air, and by the tube, which leads to the meter. The quantity of air which is drawn through it by this tube amounts to about 20,000 litres (706.4 cubic feet) per hour, a quantity not merely abundantly sufficient for ventilation, but to prevent loss or error by diffusion into the air through the chinks round the door. It is quite unnecessary to describe the aspirating apparatus, excepting in so far as to state that the clockwork is moved by a weight, which, by a well-known mechanical contrivance, is constantly wound up by a steam-engine.

To obtain a result, we must be able to determine with accuracy, first, the duration of the period of observation, and, secondly, the quantity of carbonic acid gas contained in the air which passes out of the chamber during that period. The latter object may be attained either by estimating the total weight of carbonic acid discharged, or by measuring the volume of air aspired, and, simultaneously, the proportion by volume of the same gas contained in it. In the apparatus above described, the quantity of air discharged is so large that it would not be possible to analyse the whole of it, so that the second of the two alternatives must be adopted. This is effected not by taking one or more specimens of the discharged air from time to time and analysing them (for this plan, unless a very great number of analyses were made would, in consequence of the constant irregularities which occur in the rate of discharge, give wrong results), but by causing a *definite proportion* of the used air to pass through an absorbing apparatus, and measuring the total quantity of carbonic acid gas contained in it by a volumetrical method to be immediately described. This division of the aspired air into two parts, one to be measured and analysed, the other merely to be measured, is a matter of great difficulty;

for it obviously involves the carrying on during the period of observation of two continuous measurements—*i.e.*, the employment of two meters instead of one, each of which must give results which are not only accurate in themselves, but must correspond exactly with those of the other. As in applying the method to animals so small that the whole quantity of air can be analysed, this difficulty is not met with, it is not necessary to say anything as to the means of obviating it, or the errors which, in spite of all precautions, it occasions.

98. **Application of Pettenkofer's Method to the Determination of the Discharge of Carbonic Acid Gas in Small Animals.**—The apparatus consists of a metal chamber of iron, which communicates in one direction with the Bunsen's water-air pump; in the other, with the apparatus for the absorption of carbonic acid gas. Its lid closes air-tight by means of a mercurial joint. For a guinea-pig or rat, it should have a capacity of about 500 cubic inches (8193 c.c.). Between the blower and the chamber is interposed a flask of about 6 oz. capacity, through the cork of which two tubes pass; of these, one is prolonged nearly to the bottom; the other, the exit tube, ends just below the under surface of the cork. This flask is filled with pumice, moistened with solution of potash. In this way the chamber is supplied with a constant and perfectly steady stream of air, free from carbonic acid. As, however, the quantity of air supplied by the blower is much larger than is required, it must be diminished by allowing a certain quantity to waste. For this purpose a T tube is interposed between the blower and the potash flask, the stem of which is connected by an india-rubber tube with a kind of safety-valve, the construction of which is the same as that of valve B, in fig. 251; the waste of air may be increased or diminished by raising or lowering the longer of the two tubes. The absorbing apparatus consists of two or a greater number of absorption tubes (fig. 252), which are charged with absorbing liquid. When each tube is placed at its proper inclination, and the difference between the pressure on opposite sides of the column of liquid is not too great, the air, which enters the short limb by an end of india-rubber tube which reaches nearly to the bend, passes up the long arm in a regular succession of bubbles so small that it is thoroughly acted on by the liquid. The two tubes are charged with a solution of baryta, which in the longer is three times as strong as in the shorter. The strengths of both solutions are determined volumetrically by a standard solution of oxalic acid before and after every period of observation.

Preparation of the Solutions of Baryta and Oxalic Acid.—

Of the two solutions of baryta which are in use, the stronger contains about 21 grammes of hydrate of baryta in a litre, the other 7 grammes; the former is obtained by adding sufficient distilled water to 420 cub. cent. of saturated baryta water to make up a litre; the latter contains 140 cub. cent. in a litre. These liquids must be kept in bottles which have no communication with the air, excepting through a flask or absorption tube filled with pumice, moistened with potash. The oxalic acid solution must be prepared with the utmost accuracy. It must contain 2·8636 grammes of pure well-crystallized oxalic acid, free from efflorescence, in a litre. Before making the solution, it is necessary to dry the crystals over sulphuric acid for a few hours. The strength of this solution stands in the ratio of exactly 1 to 22 to that of the ordinary volumetrical solution of the pharmacopœia. It keeps badly, being apt to become mouldy, so that if a large amount is required, it is better to keep the weighed quantities of oxalic acid than the liquid.

Mode of determining the Strength of the Baryta Solution.—Thirty centimeters of baryta water having been introduced into a small flask, the solution of oxalic acid is cautiously added from a finely graduated burette. Between each addition, the flask is closed with the thumb and shaken. As an indicator, Pettenkofer has found that turmeric paper gives better results than litmus. The paper must be prepared by digesting turmeric root in weak alcohol, and dipping strips of Swedish filter paper into it, which must then be dried in a dark place, and kept in the dark. When the liquid is so nearly neutralized that it does not brown a strip of paper dipped into it, a drop is placed with a rod on the strip. If there is still a trace of alkaline reaction, a brown line appears at the periphery. As soon as this is no longer the case, the point of complete neutralization has been attained. This reaction is so delicate, that it is sensibly affected by the presence of one-tenth of a cubic centimeter of solution of oxalic acid, *i.e.*, one-tenth of a milligramme of carbonic acid gas, so that the results of two determinations of the same liquid ought not to differ from each other by more than the quantity named. It is well, in order to save time, to make a first experiment with a small quantity (say 5 cub. cent.). It is of great practical importance to notice that the baryta solution must contain no trace of caustic potash, or soda, the smallest quantities of which render the determination impossible—for the oxalate of potash or soda formed in this case re-acts on the carbonate of baryta present, so as to produce oxalate of baryta and carbonate of soda. Consequently, the liquid never loses its alkaline reaction, for each renewed addition of oxalic acid re-converts the alkaline carbonate into oxalate, which is again decomposed by the carbonate of baryta as before.

Mode of preparing and filling the Absorption Tubes.—The short arm of each tube is filled air-tight with an india-rubber cork, pierced with a tube. The larger tube is connected at its opposite end with the smaller, from which the air finally escapes through an india-rubber tube, guarded by a screw-clip. By adjusting this clip, the size of the bubbles is regulated, their magnitude varying inversely as the resistance. To fill the tubes, the required quantities of liquid are introduced into flasks, fitted with air-tight corks, having necks sufficiently wide for the introduction of a pipette. The strength of the solution having been determined in thirty centimeters, as above described, the stronger solution is to be delivered into the first absorption tube in three quantities of 45 centimeters, and two such quantities of the weaker into the second. [Tubes of the size required for these quantities are made by Cetti & Co., of Brooke Street, Holborn.] The tubes are then closed and adjusted to the proper inclination (previously ascertained by trial). At the close of the experiment, the liquids are transferred once more to flasks, similar to those above described, and their strength determined as before. The calculation of the result is simple. The quantity of carbonic acid absorbed by each 30 cub. cent. of the liquid is indicated by the difference between the corresponding quantities of oxalic acid solution used, before and after absorption. This quantity must be multiplied in the one case by $\frac{135}{30} = 4{\cdot}5$, in the other by $\frac{90}{30} = 3$. The sum of the two products is the total quantity of carbonic acid disengaged during the period of observation. If an animal larger than a guineapig is used, it is necessary to employ two sets of absorbing tubes, or a greater number.

SECTION V.—INNERVATION OF THE RESPIRATORY MOVEMENTS.

The rhythmical movements of respiration depend on the activity of a centre contained in that part of the floor of the fourth ventricle from which the roots of the vagus nerve spring. The proof of this fact lies in the fundamental experiment of Legallois, by which he showed that the cerebrum, the cerebellum, and even part of the medulla oblongata itself may be removed, without arresting respiration. This experiment has already been described in § 92.

By motor nerves this centre is in relation with the muscles of which the combined rhythmical actions have been studied in the same paragraph. Its discharges of energy, like those of the motor centres of the heart, are automatic, but their rhythm is constantly subject to modification by impressions received through the afferent fibres of the vagi. Consequently, the study of the innervation of the respiratory movements resolves itself into experiments relating to the respiratory functions of these nerves. The results of such experiments may be divided according as they relate to the effects of section of both vagi, to excitation of the central end of the divided nerves, or to excitation of the superior laryngeal nerve.

99. Section of both Vagi in the Neck.—In the paragraph relating to the functions of the vagus as a heart-nerve, directions have been given as to the mode of preparing it. The rabbit is preferable to the dog or cat, for in those animals the vagus is united in one trunk with the sympathetic. Section of the vagi is the simplest and at the same time one of the most instructive experiments relating to the physiology of the nervous system. The animal having been secured in the usual way on Czermak's rabbit support, a ligature is passed round each nerve a little below the cricoid cartilage. The ends of each ligature are then knotted together, so as to facilitate their being found at any moment. To observe the effect, the animal should be placed before and after section, under the same circumstances. If it is not intended to record the results graphically, it may be allowed to run about while the respirations are counted, and careful observations are made as to the respiratory movements. For more exact observations various methods may be used, each of which is of some value. The first consists in recording the movements of the diaphragm on the kymograph, as directed in § 91.* The second and third are so contrived as to show not only the duration and rhythm of the respiratory movements before and after section, but the extent of the respiratory exchange of air. The apparatus for this purpose is constructed as follows: A large bottle, capable of holding five gallons or more, is closed air-tight with an india-rubber cork, into which the stem of a glass **T** tube is carefully fitted. Of the two branches of the **T** tube, one communicates with the respiratory cavity of the animal, by a connector of india-rubber and a glass cannula secured air-tight in the trachea, the other is left open and can be readily closed with the finger. The bottle also communicates by a second glass tube which passes through its india-rubber stopper, with a Marey's tympanum, the lever of which writes on the blackened cylinder of the kymograph. This tube is controlled by a screw clamp. So long as the arm of the **T** piece is left open, the animal of course breathes the external air freely. On placing the finger against the aperture, it begins to breathe the air of the bottle, but inasmuch as the capacity of this vessel is 250 times as great as the respiratory cavity of the rabbit, it can do so for some time without the slightest dyspnœa, as is proved by the observation that the depressors of the larynx do not come into action. The resistance is, however, sufficiently great to affect the lever of the tympanum, the rise and fall of which in each respiratory act is in exact proportion to the quantity of air breathed. The animal having been chloralised and both nerves prepared as above described, a few tracings should be taken of the normal respiration. This done, the clockwork is again set in motion and both nerves are divided at the same moment. In this way a tracing (fig. 253) is obtained, which strikingly exhibits the effects of section, both as regards the rhythm and extent of the thoracic movements.

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* The tracing so obtained is shown in the first (normal) part of fig. 255.

Another method consists in measuring the quantity of air inspired in a given time with the aid of the apparatus shown in fig. 251. In this way the effect of section on the respiratory exchange can be estimated with much greater precision than in any other, but obviously no information is obtained as to the respiratory movements.

100. The most important results are as follows : 1. In the adult rabbit, the number of respirations per minute diminishes from 120—140 to 40—50. That this is only to a very slight extent dependent on the narrowing of the glottis due to the relaxation of the intrinsic muscles of the larynx, is proved by the fact that if the two recurrents are divided, the retarding effect of the operation is very inconsiderable, while the retarding effect of section is diminished in no appreciable degree by previous tracheotomy. 2. The mechanism of breathing is completely altered. Each respiration is about five times as deep as it was before. This depends partly on increased action of the diaphragm, partly on the participation of the accessory muscles in the inspiratory act. The belly is projected and the larynx drawn down by the sternal muscles in each inspiration, while the upper ribs, which before were motionless, are drawn upwards and outwards by the external intercostals and intercartilaginous muscles. The inspiratory expansion of the upper part of the thorax lasts for several seconds, at the end of which it suddenly collapses, expelling the air with such force as to make an audible sonorous noise in the air passages, often accompanied, if the trachea has not been opened, with a coarse râle. This sudden collapse, which is a non-muscular act, is followed by a long pause, the existence of which is characteristic. At the end of it there usually occurs a short expiratory movement, attended with hardening of the muscles of the abdominal wall, which is the immediate precursor of the inspiratory act.——The mode of breathing just described is that of dyspnœa; but there is this important difference between ordinary dyspnœa and that produced by section of the vagi, that whereas in the former the

frequency of the respiratory movements is increased, in the latter it is diminished; with this exception, all the characteristics of dyspnœa are present. 3. The quantity of air breathed per minute is as great after section of both vagi as before, the diminished frequency of the respirations being counterbalanced by the increased depth of the respiratory act. This is proved by measuring the quantity of air breathed in a given time in the manner above directed. 4. These facts afford ground for inferring that although section of both vagi does not materially either increase or diminish the work done in a given time by the respiratory muscles, it interferes very considerably with the accomplishment of the purpose of their movements,—the arterialization of the blood. Notwithstanding the vigour of the respiratory movements, the blood becomes more or less venous.

101. Death after Section of both Vagi.—Rabbits in which both vagi have been divided, commonly die before the end of the first day. Dogs live longer—often two or three days. After death, the lungs are found in an altered condition, of which the following are the leading features: The mucous lining of the air passages is reddened (especially in dogs), the colour being due to the injection of the capillaries of the mucosa with blood. The lungs collapse much less than naturally when the chest is opened. The pulmonary parenchyma is, to a greater or less extent, devoid of air. The airless parts are soaked with a brownish-red serous liquid, and here and there choked with a greyish white material, which, on microscopical examination, is found to consist of young cells (pus-corpuscles). Similar cells are seen in the serous liquid along with numerous blood corpuscles. These changes may be accounted for as follows:—When the vagi are divided, all the parts to which the branches below the point of section are distributed are affected, *e.g.*, the larynx, air passages, lungs, œsophagus, &c. 1. The glottis is partially closed, just as it is after death. 2. The mucous lining of the air passages is deprived of sensibility, so that, when it is irritated, no cough is produced. 3. The muscular fibres of the œsophagus are paralyzed, so that regurgitation of food from the stomach is apt to take place; the muscular fibres of the bronchial tubes are in a similar condition. With reference to these co-efficients in the production of the lung affection we have the following facts, showing that the first two are at all events the only ones which are of importance:—(*a*) A lung affection of the same nature as that induced by section of both vagi, though of inferior intensity, follows section of the inferior laryngeal nerves. (*b*) In animals with divided vagi, life is prolonged by tracheotomy, the degree of prolongation depending on the efficiency of means used to prevent the entrance of foreign bodies into the air passages.

(*c*) In animals of which the vagi are intact, a lung affection is produced by injecting mucus from the pharynx into the air passages which is of the same nature with that now under consideration. The combination of these facts leads to the inference that the inflammation of the lungs of which animals with divided vagi die, is dependent on the intrusion of foreign bodies from the pharynx into the air passages and lungs, rather than to any direct effect of the section on the lung-tissue.

102. Demonstration of the Respiratory Functions of Afferent Fibres of the Vagus, by Excitation of the Central End of the Divided Nerve.—The method of preparing the nerve has been already described. The excitor, shown in fig. 225, is used. It is better to employ Helmholtz's side wire (*see* next paragraph), but not necessary; for even when strong unmodified induced currents are used, there is little danger of unipolar effects, the extent to which the nerve can be separated being such, that there is no difficulty in interposing a considerable air space between it and the surrounding parts.

The phenomena which accompany excitation of the central end of the divided vagus vary according to the state of the animal and the state of the nerve. It will be convenient to describe them under heads corresponding to these conditions:—1 *Animal breathing naturally.* To observe what may be regarded as the normal results of excitation, care must be taken that the subject of experiment is not exhausted, and that, in placing it on the support, nothing is done which can interfere with its breathing. The movements of the diaphragm must be recorded * either with the aid of the apparatus, fig. 250, or in the manner described in the preceding paragraph; but for the present purpose, by far the best method is to introduce into the peritonæal cavity, by means of a small opening in the linea alba close to the ensiform cartilage, a small flat bag of india-rubber, of such size that it can be conveniently slipped between the diaphragm and liver. If this bag is slightly distended with air and connected with a Marey's tympanum, it gives excellent tracings of the diaphragmatic movements. To the student who witnesses the experiment for the first time, a still more convincing mode of appreciating the effect of exciting the central end on the diaphragm is to feel the contraction of the muscle with the finger during the period of excitation. The nerve having been prepared, and the excitor placed under it, a preliminary tracing must be taken of the normal respiration. In a tracing, taken by the method described in § 99, it is seen that in each respiratory act three parts may be distinguished, one of which, the ascent, expresses inspiration, or active contraction of the diaphragm; the whole of the remainder of the period corresponds to relaxation of that muscle. Sometimes the part of the curve which immediately precedes the ascent indicates that towards the close of the period of relaxation air neither enters nor leaves the chest. If a straight line is drawn through the angle formed in each curve at the point corresponding to the commencement of inspiration, it may be taken as indicating the position of the lever when the diaphragm is at rest after an ordinary expiration. So long as air is passing out of the chest, the lever keeps below this line, but as soon as the outflow ceases, provided that the diaphragm is still relaxed, it returns to it. Hence the line corresponds to the position of equilibrium. These facts are well seen in the first (normal) part of tracing, fig. 255.

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* *See* fig. 254 *a.*

The tympanum having now been connected with the bag between the diaphragm and liver, as above described, and the secondary coil placed at a considerable distance from the primary, the key which has been connected with the telegraph is opened. The effect cannot be predicted with certainty. Probably the respiratory movements will be quickened, the lever assuming a somewhat higher position during the period of excitation than it did before. This indicates that the diaphragm descends further in each inspiration, and does not relax quite so much in expiration.

The secondary coil must now be gradually brought up nearer, while the excitation is repeated after each shifting, until it is observed that the lever ascends and remains stationary each time the key is opened, drawing a nearly horizontal line at a much higher level than that of the previous part of the tracing. (*See* fig. 254*b*.*) If the excitation is continued only for a few seconds, the elevation of the lever which indicates contraction of the diaphragm not only continues during the whole time, but lasts a second or two after it. The lever then gradually falls, and after a few moments resumes its up-and-down movements, always beginning with a descent. In other words, the diaphragm, after a period of contraction, which somewhat exceeds its cause in duration, is for a moment relaxed before it resumes its rhythmical action. The conduct of the other respiratory muscles should be carefully watched (by another observer) during these experiments. It will be seen that, provided that the animal is breathing perfectly naturally at the moment that the key is opened, the descent of the diaphragm determined by the excitation of the vagus is not attended by any other muscular movement, and, in particular, that the upper ribs remain as motionless as before, and that the larynx does not descend.* 2. *Animal in the state of apnœa.* In a rabbit of which the blood has been surcharged with oxygen by excessive artificial respiration, the effect of exciting the central end of the vagus is negative. No respiratory movement is produced. To demonstrate this, experiments must be made before, during, and after apnœa. It is found that the same current which tetanizes the diaphragm in the normal state, has no effect when the blood is over-arterialized. This is an experiment of fundamental importance, because it shows that the relation between the vagus and the motor nerves of respiration (and particularly the phrenic) is entirely different from that which exists between the afferent and efferent nerve in the ordinary case of reflex action. 3. *Animal in the state of dyspnœa.* When the blood, instead of containing too much oxygen, contains too little, the effect of excitation of the central end extends itself to all the extra muscles which are at the time in action; consequently, the greater the dyspnœa, the greater is the number of muscles which respond to the stimulus. This is best seen in an animal, in which, after perforation of one side of the chest, respiration is maintained artificially; the same rabbit which has served for the other experiments may be used. The result may be varied according to the degree of dyspnœa produced, by regulating the frequency and quantity of the injections of air. If, for example, the dyspnœa is sufficient to bring into action the external intercostals, intercartilaginei, and scaleni, all these muscles contract simultaneously with the diaphragm when the central end is excited, so that the chest remains, during the time that the key is open, in a state of tetanic expansion.

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* Fig. 254*b* shows that during the whole period of excitation (indicated by the horizontal line below) the diaphragm remained contracted; then followed a few irregular movements, after which the rhythmical movements were resumed with a slightly increased frequency. The period of contraction was interrupted, as frequently happens, by a momentary relaxation.

103. *Excitation of the Central End of one Vagus after Section of both.*—By very careful graduation of the induced current (with Helmholtz' modification), it is sometimes possible to supply the precise degree of excitation to the vagus centre, which is required to make up for the loss sustained by the section of its afferent fibres, and in this way to restore the normal respiratory rhythm. More frequently the experiment fails, and effects are produced which correspond to those described above.

Exceptional Cases.—It very frequently happens, particularly in animals under the influence of chloral, that effects are produced by excitation of the central end which are just the opposite of those which we regard as normal. The diaphragm, instead of contracting, relaxes, and remains relaxed during the whole time (*see* fig. 255 *) that the key is open. The immediate cause of this generally is that the nerve is exhausted. The reason why it happens is that the vagus contains (in addition to the fibres which, when excited, act on the vagus centre in such a way as to lessen the hypothetical resistance by which it is normally prevented from discharging itself in muscular contractions) other fibres, which in the language of physiologists are "inhibitory," *i.e.*, tend to increase the resistance above referred to. In the fresh state of the nerve, the influence of these fibres is completely overbalanced by that of the others. In the exhausted state, this relation is reversed, so that the two sets of afferent fibres are as much distinguished from each other by their difference of endurance, as by their differences of function. Recent experiments (Burkart) make it probable that the "inhibitory" fibres come mostly from the recurrents.

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* Fig. 254*a* was obtained in the same animal as 254*b* with the aid of the apparatus, fig. 250. The tracing shows that the rhythmical movements were not resumed until a second or two after excitation had ceased. They were at first somewhat more frequent than before, and the diaphragm was in a lower position. In less than a minute the previous conditions were restored in both respects.

104. Excitation of the Superior Laryngeal Nerve.—The experimental investigation of the superior laryngeal is much more difficult than that of the trunk of the vagus, partly because the nerve is difficult to reach and runs a short course, partly because it is very slender. To expose it in the rabbit, an incision should be made extending from the side of the trachea at the level of its first and second rings to the hollow between the angle of the jaw and the larynx. After severing the skin in the usual way, the fascia which extends forwards from the edge of the sterno-mastoid muscle must be carefully broken through with the aid of two pairs of dissecting forceps, so as to expose the parts seen in fig. 227. The space is divided into two by the artery, the direction of which coincides exactly with that of the original incision. Near its lower end the artery gives off its thyroid branch. At the top the space is limited by the tendon of the stylohyoid muscle, and the posterior cornu of the hyoid bone. Immediately below the muscle is the trunk of the ninth nerve which arches forwards towards the tongue. The descending branch of that nerve passes downwards and forwards to reach the muscles which cover the front of the trachea, giving communicating branches to the cervical plexus, and a branch which arches forwards over the artery to gain the muscles which draw the larynx upwards.—Before proceeding to expose the deeper nerves, it is well, in order to avoid confusion, to remove the *descendens noni:* the next step is to draw the larynx well to the side opposite to that chosen for the incision, so as to widen the space between it and the carotid artery. This done, the exposure of the superior laryngeal becomes easy. Its exact position is indicated in the figure; its course is much twisted so as to allow of the up-and-down movements of the larynx. In preparing it, no cutting instruments must be used. It must be freed from the surrounding structures with the aid of two pairs of forceps, any veins in the way having been divided between two ligatures. Care must be taken, however, to leave a certain quantity of cellular tissue about it to serve as a kind of protective sheath, and make it somewhat less liable to get dry. The nerve having been prepared, a ligature must be tied round it as near as possible to the thyrohyoid membrane, after which it must be divided beyond. In the dog or cat the mode of preparation is very much the same as in the rabbit. In the cat, the comparative thickness of the nerve facilitates the manipulation.

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* The tracing, fig. 255, was obtained by the method described in § 99. During the whole period of excitation the diaphragm remained stationary in the position of ordinary expiration; almost immediately after, the rhythmical movements were resumed, the first movement being an ordinary inspiration. The period was interrupted by a single respiratory movement caused by the accidental removal of the electrodes from the nerve. The notches in the horizontal part of the tracing express cardiac pulsations.

In exciting the superior laryngeal, the great source of difficulty is the proximity of the vagus and the consequent liability of that nerve to be acted on by the induced current in a unipolar way. This accident, which is of course fatal to the success of the investigation, the functions of the two nerves being opposite, is to be avoided, not by the use of complicated arrangements for the insulation of the nerve, but by placing it in such a way on the ordinary copper points that the part acted on is separated by a considerable air space from the surrounding tissues. Before beginning the excitation, the secondary coil must be shifted to a distance from the primary, and the primary current divided by means of Helmholtz's side wire into two branches, one of which only passes through the breaker. The other is led directly from the battery to the coil, so that the primary current is never entirely opened. In this way the opening induction shock, which, in the ordinary arrangement of the induction apparatus, possesses a much greater tension than that of the closing shock, is so reduced that the two become nearly equal to each other.* Consequently, as the risk of unipolar action varies with the maximum intensity of the current, it is very much diminished by this contrivance—so much so, indeed, that if care is taken to prepare the nerve properly, even moderately strong currents may be used without any effects referable to unipolar excitation of the vagus manifesting themselves.—Excitation of the central end of the superior laryngeal produces, according to the strength of the current used, either diminution of frequency of the respiratory movements or complete relaxation of the muscles of inspiration. The most advantageous way of judging of its effect on the diaphragm, is to expose that muscle in the way directed in § 91. It is then seen that that muscle becomes absosolutely flaccid during excitation of the nerve, and it is drawn up by the elastic contraction of the lungs, so as to assume its highest possible position. When the excitation is discontinued, the relaxation either gives way to natural breathing or is immediately succeeded by one or two vigorous inspirations. If the current is so feeble that it merely diminishes the frequency of the respirations, without arresting them, the tracings show that there is no diminution of the duration of the inspiratory acts, and that the slowing is entirely due to a prolongation of the intervals, *i.e.*, of the periods during which the diaphragm remains in the position assumed by it at the close of ordinary expiration. To record the effects graphically, any of the methods recommended in the preceding paragraphs may be used. If the method described in § 99 is employed, a tracing is obtained which exactly resembles fig. 255. The tracing, fig. 256,* was drawn by inserting a bag between the diaphragm and the liver.

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* For a fuller explanation of the difference between the two induced currents and of the effect of Helmholtz's modification, *see* Rosenthal, "Electricitätslehre," p 120.

SECTION VI.—INFLUENCE OF THE RESPIRATION ON THE CIRCULATION.

105.—If the stethoscope is applied to the præcordia of a dog, it is easily observed, especially if the animal has been narcotized, that the rate at which the contractions of the heart succeed each other is subject to rhythmically recurring variations, and that the acceleration follows each expansion of the chest, lasting during the first part of the succeeding expiration; while during the latter part of the expiratory period—the period during which, as we have seen, air is expelled very slowly—the diastolic intervals become longer. These facts admit of much more precise demonstration by the graphic method. For this purpose the most convenient instrument is that shown in fig. 257.

It is a kymograph so constructed as to record the arterial pressure and respiratory movements simultaneously. The mercurial manometer consists of two limbs of equal length, one of which, the distal (A), is much wider than the other near the top, the relation between the lumen of the one and that of the other being 1:10. The float which rests on the distal column is of box-wood. Its under surface is concave, so as to fit the convex surface of the mercury. By the vertical rod it is connected with a light lever, D, about two feet in length, which is counterpoised by a weight suspended to it on the other side of the brass bearing, E. At its thin end, the lever carries a pen, the distance of which from D is such, that for every inch of variation of difference between the two columns of the manometer, it rises or falls three-tenths of an inch. It will be readily understood that the movement of the pen, instead of being rectilinear, is circular; consequently, it is vertical only when the lever is horizontal; for which reason the fulcrum, E, which is so constructed as to slide up and down on the brass uprights, must always be placed in such a position that the lever is horizontal. The height of the mercurial column corresponds to the average arterial pressure. That part of the instrument which is intended for recording the respiratory movements, consists of a Marey's tympanum, C, and a lever, F, similar to D and of the same length, with which it is connected. The tube, H, of the tympanum may be either brought into communication with one arm of a glass T tube, the stem of which is inserted in the trachea, or with a stethometer applied to the chest. The lever of the tympanum is connected with the recording lever by a vertical rod seen in the drawing. In this way two tracings are obtained simultaneously, of which fig. 258, is an example. The arterial tracing is marked A P, the respiratory R. In the latter, the beginning of inspiration is indicated by the vertical stroke *a;* of expiration by *b;* of the pause by *c*. The coincident points in A P are indicated by similar strokes. The break is made by removing both pens from the paper by the same act. In man, the variations of frequency (which of course can alone be investigated) are absent in most healthy persons, although very obvious in certain conditions of disease. In the rabbit they are much less marked than in the dog. They are regarded by most physiologists as dependent on variations of activity of the intracranial centre of the cardiac vagus: until very recently it has been assumed, by way of explanation, that the respiratory movements affect the cerebral circulation in such a way that during the period of relaxation of the muscles of respiration, the supply of blood to the medulla oblongata is diminished, and increased during their contraction—and that the inhibitory nervous system of the heart is affected by these changes. This explanation has always appeared unsatisfactory, and could only be accepted provisionally; for it seemed extremely improbable that there was any appreciable difference in the supply of blood between the inspiratory and expiratory periods. We now know that the respiratory variations in the arterial pressure and in the frequency of the contractions of the heart, are not necessarily dependent on the mechanical effect of the respiratory movements on the heart, inasmuch as they persist when these movements are abolished; and that they have their primary source in the vasomotor and cardiac-inhibitory centres, which act rhythmically, not because they are subject to any rhythmical excitation, but because they have periods of waxing and waning activity which correspond to those of the respiratory centre. A very little consideration shows that this inference carries the admission, that the cardiac-inhibitory centre and the vasomotor centre act alternately, for it can be seen in every tracing that the increase of arterial tension determined by increased vascular tonus, alternates with the retarded pulse and diminished tension produced by "vagus excitation." In other words, the phase of maximum activity of the inhibitory centre always coincides with that of minimum activity of the vasomotor centre. The experiment by which it is proved that the respiratory phases of arterial pressure and pulse frequency are independent of the thoracic movement, consists in curarizing a dog by the injection into the venous system of a dose of solution of curare only just sufficient to paralyse the respiratory muscles (5 to 10 millig. for a dog of 10 lbs. weight), and observing graphically the changes of arterial pressure which occur during the gradual extinction of the respiratory movements, with the aid of the apparatus described above. The tracings, figs. 259–261, show what is observed at three different stages of curarization. Curve 259 was drawn when the animal's muscles were still active. It may be regarded as normal. Curve 260* corresponds to a period at which each inspiration and expiration is represented by a scarcely perceptible contraction and dilatation of the chest. Curve 261 to a still later condition, in which the inspiratory movements are indicated by a mere vibration of the lever, produced (as was observed at the time) by momentary contraction of certain inspiratory muscles which were not yet completely paralysed. We learn from these observations, that during the gradual extinction of the respiratory movements, the intervals between them correspondingly lengthen; and that at first the variations of arterial pressure and pulse frequency exhibit the characters which closely correspond to those they exhibit normally. Subsequently, the ascents and descents of the mercurial column become much more gradual, and the changes of frequency less abrupt. Finally they assume, so far as relates to arterial tension, the characters of the variations known as Traube's curves, to be described in the next paragraph.

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* The tracing, fig. 256, shows that during the whole period of excitation the diaphragm remained motionless in the position of expiration, with the exception that at gradually lengthening intervals it executed momentary contractions. When, after the cessation of excitation, the respiratory movements were resumed, they were slower but more extensive than before.

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* In fig. 260 the notches in the lower tracing represent rudimentary inspirations and expirations. The expiratory movements, *e e e*, are only traceable, however, in the last half of the tracing; they follow the inspiratory, *i i i*, at an interval of about five mill. = 1½ sec. In fig. 261, the expiratory movements are wholly indistinguishable. All the tracings of this series are reduced one-half to save space. The distance between the respiratory and arterial tracing is also diminished for the same reason.

106. Traube's Curves.—This term is applied by physiologists to the rhythmical variations of arterial pressure which occur in curarized animals, after complete cessation of the respiratory movements, and section of both vagi. They can be demonstrated in the rabbit, cat, or dog, but most readily in the last. Traube described them as they occur in the absence of artificial respiration, *i.e.*, when the inflations are for a time discontinued. During the gradual rise of arterial pressure which, as we have aready seen, takes place under those circumstances, the arterial pressure-curve exhibits the undulations in question. It has, however, been lately shown by Hering, that the state of asphyxia is far from being essential, and that the most certain way of producing the phenomenon is to bring the blood of the animal into a state which corresponds to dyspnœa, not by stopping the artificial respiration altogether, but by gradually diminishing the quantity injected at each stroke. In the arterial tracings so obtained it is seen that the cardiac intervals are of uniform duration—in other words, that there are no variations of pulse-frequency, the vagi having been divided. When these nerves are left intact, curves are obtained (fig. 262 *) in which the variations of the pulse intervals exhibit the same relation to those of the arterial tension as in the normal condition—the pulse frequency being greater in the ascending limb of each respiratory wave than in the descending. From this we learn that the variations of frequency are dependent on the integrity of the vagi.—The proof that the variations of pressure are vascular in their origin, and depend on corresponding changes of arterial tonus, is shown by two experimental results, viz.: (*a*) that although after section of the spinal cord, arterial pressure is still subject to variations which are no doubt dependent on changes of arterial tonus, these are very irregular; and (*b*) that the rhythmical variations of pressure persist after the influence of the heart has been eliminated. The latter fact has been demonstrated by Hering, who has shown that if circulation is maintained artificially, independently of the heart, in an animal which is placed in other respects in conditions favourable to the production of "Traube's curves," they exhibit themselves with the same distinctness as when the heart is in action.

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* The tracings, fig. 262, are those of a curarized dog with *undivided* vagi, in which air is injected into the lungs at regular intervals, but in insufficient quantity. The arterial curve differs from "Traube's" only in this respect—that in the ascending limb of each wave, the wavelets which express the arterial pulsations are more frequent than in the descending. In the lower tracing the ascents mark the strokes of the artificial respiration apparatus, which was working at intervals of five seconds: the variations of arterial pressure shown in the upper tracing follow the rhythm of the natural respiratory movements, and consequently do not correspond with the inflations.

The conclusion to be derived from the preceding experiments may be expressed as follows: The rhythmical variations of arterial pressure which are associated with the respiratory movements, are dependent on corresponding variations of arterial tonus, but the variations of the frequency of the contraction of the heart are governed by the inhibitory nervous system of that organ. In accepting this proposition, it must not be forgotten that under normal conditions the thoracic movements co-operate in the most powerful manner in the production of the result; for in every inspiration, so long as the pleural cavities remain closed, the diastolic impletion of the heart is favoured by the filling of the venæ cavæ, and thereby the vigour of the succeeding contractions of the heart is increased. This is particularly the case in animals which (like the dog and cat) breathe thoracically.

SECTION VII.—APNŒA, DYSPNŒA AND ASPHYXIA.

The terms apnœa, dyspnœa, and asphyxia, are applied in physiology to the states of functional disorder which are produced by excess and defect of oxygen in the blood, the differences between them being,—in accordance with a generalization so well established that it may be regarded as a law,—that the activity of the respiratory movements varies inversely as their effect on the blood.

107. Apnœa.—When the blood is saturated with oxygen, respiratory movements cease, and the animal is said to be in a state of apnœa. The fact can be demonstrated with great ease in the rabbit by the ordinary method of artificial respiration. If the intervals between the inflations of the lungs are gradually shortened, the inspiratory movements become shallower and shallower, and finally cease. The heart continues to beat vigorously and somewhat more frequently than before. The visible mucous membranes present a perfectly natural appearance. The eye closes instantly when the conjunctiva is touched, and the state of the pupil is normal. In short, all the functions excepting the respiratory movements go on as before.*

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* The fact of apnœa was first demonstrated by Hook, before the Royal Society in October, 1667. His experiment consists in opening the chest of a dog, distending the lungs with bellows, and keeping up a constant stream of air through the organ through punctures made in its surface for the purpose. He found that, although "the eyes were all the time very quick, and the heart beating regularly," there were no respiratory movements. The term "apnœa" was first applied to this condition by Rosenthal, in 1864.

108. Dyspnœa.—We have already studied the phenomena of dyspnœa so far as relates to the muscular movements. We have seen that in the rabbit, when the access of air to the circulating blood is gradually diminished, other muscles begin to co-operate with the diaphragm in the inspiratory act, in an order which, as a rule, is as follows:—*Intercostales externi, levatores costarum breves, intercartilaginei, scaleni, serrati postici.* As external signs of dyspnœa, the drawing down of the larynx in inspiration by the muscles which cover the trachea, and the expansion of the upper part of the chest by the intercartilaginous muscles and external intercostals, are the most important, as indicating successive stages.—For a comprehensive study of dyspnœa, as it affects not merely the respiratory movements, but the circulation and the functions of the nervous system, those experiments are best in which the disorder can be watched from its beginnings in mere increase of functional activity (hyperpnœa), to its issue in asphyxia or suffocation. These may consist, either in complete obstruction of the air passages, in which case death occurs very rapidly (in 4-5 minutes in the dog, a shorter time in the rabbit), in allowing the animal to breathe out of a bag with which its respiratory cavity is in air-tight communication, or from a gasometer (the instrument represented in fig. 251), into which definite mixtures of gases can be continuously introduced.

109. Asphyxia by complete Occlusion of the Trachea.—For this purpose, a cannula must be fixed air-tight in the trachea, the mouth of which is of such form that it can be plugged with a cork. If it is desired to obtain a tracing of the variations of tension which the air so enclosed in the respiratory cavity undergoes, the cork must be perforated and fitted with a tube which communicates with a mercurial manometer, the movements of which are recorded on the cylinder of the kymograph, simultaneously with the variations of pressure in the crural artery. The tube must be of small bore and have thick walls. The phenomena, as they present themselves in the dog, may be enumerated as follows: *First minute.*—Excessive respiratory movements, in which at first the expansive efforts of the thoracic muscles, afterwards the expulsive efforts of the muscles of the abdominal wall, are most violent. During this period the arterial pressure increases, but it is extremely difficult to measure it, on account of the modifying influence of the thoracic movements. Towards the close of the first minute the animal becomes convulsed. These convulsions must be attentively studied, because they are the type, by comparison with which all other convulsions of the same order are described or defined. The prominent fact with respect to these convulsions is that they are expiratory. At first, indeed, they seem to be nothing more than exaggerations of the previous expulsive efforts. Afterwards, the contractions of the proper expiratory muscles are accompanied by more or less irregular spasms of the muscles of the limbs, particularly of the flexors. *Second minute.*—Early in the second minute the convulsions cease, often suddenly; simultaneously with their cessation, the expiratory efforts become indistinguishable. The iris is now dilated to a rim; the eye does not close when the cornea is touched, nor does the pupil react to light; all reflex reaction to stimuli has ceased. All the muscles, except those of inspiration are flaccid, and the animal lies in a state of tranquillity, which contrasts in the most striking way with the storm which preceded it. The condition of the circulation at this stage can be best judged of by the tracing, fig. 263*b*.* Inspirations occur at long but tolerably regular intervals, and each inspiratory act is accompanied, not, as in normal inspiration, by an increase of arterial pressure, but by a marked diminution. The *mean* arterial pressure, which at the beginning of the second minute is far above the normal, sinks considerably below it towards the end. *Third and fourth minutes.*—As death approaches, the thoracic and abdominal movements, which are entirely inspiratory, become slower and slower as well as shallower. The diminution of frequency is, however, never uniform, the inspirations occurring, for the most part, in successions of two or three efforts, with long pauses between them. In each act the accessory muscles of inspiration co-operate with the diaphragm in the production of the result, and towards the close other muscles come into spasmodic action which are not usually regarded as inspiratory muscles at all, although, in all probability, they act by virtue of motor impulses originating in the inspiratory centre. In these spasms which accompany the final gasps of an asphyxiated animal, the head is thrown back, the trunk straightened or arched backwards, and the limbs are extended, while the mouth gapes and the nostrils dilate. They are called by physiologists stretching convulsions, and must be carefully distinguished by the student from the expiratory convulsions previously described.

110. Asphyxia by Slow Suffocation.—When an animal is allowed to breathe the same quantity of air repeatedly and continuously out of a bag, the process being of much longer duration, the phenomena can be studied with greater facility. As, however, its duration depends on two variable conditions, viz., the respiratory capacity of the animal and the capacity of the receptacle from which it breathes, it is not possible to describe the phenomena with reference to periods of fixed duration. It is sufficient to divide the process into two stages, the limits of which will be readily understood from the preceding paragraph. The first may be called that of hyperpnœa. The respiratory movements, at first natural, are gradually exaggerated, both as regards their extent and frequency, while the arterial pressure rises. Towards the end of the period, as in the former case, the expiratory movements gain in vigour, both absolutely and relatively to those of inspiration, so that each inspiratory act is immediately followed by a sudden tightening of the anterior abdominal wall, accompanied by convulsive twitchings of the limbs. The second stage begins by a change in the phenomena, quite as marked as when the exclusion of air is complete. Suddenly, the violent expulsive efforts cease and the inspiratory movements assume the character already described, consisting in spasmodic contractions of the diaphragm, accompanied by gasping movements of the head and neck, the most marked difference being that the arterial pressure, instead of sinking with each inspiratory effort, rises, the rise being accompanied by an equally considerable acceleration (*see* fig. 263*a*[*]). In the dog this phenomenon is so obvious that it can be judged of quite as well by watching the mercurial column of the manometer as by the tracing. As regards the gradual diminution of the frequency of the contractions of the heart during the first part of the period of collapse, and their gradual acceleration as extinction approaches, the phenomena are the same whatever be the mode in which asphyxia is produced. As regards the final respiratory movements, and the stretching convulsions which are associated with them, nothing need be added to the description previously given.

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* Fig. 263*b* is taken toward the end of the second minute of asphyxia by occlusion. The mean arterial pressure is gradually sinking; each inspiration is accompanied by a depression of arterial pressure.

The preceding facts may be summed up as follows:—In the first stage of asphyxia (understanding by the term, that part of the process which culminates in the struggle), the phenomena are of two kinds. At first, we have merely over-activity of the respiratory apparatus (hyperpnœa); at the end, expiratory convulsion. The convulsive movements are so distinct from those proper to expiration, that we are compelled to assign their origin to a special centre. This centre is often called the "convulsion centre." It is probably identical with that from which the co-ordinated expiratory movements of dyspnœa (hyperpnœa) spring; for in asphyxia we see that these last pass into convulsions by insensible gradations.[†] When the struggle with which the first stage closes is succeeded by the calm of the second, all voluntary muscles, excepting those which are either inspiratory or associated in their action with inspiration, become relaxed. The inspiratory muscles, on the contrary, act with great vigour.

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[*] Fig 263*a* is taken at the beginning of the second stage of slow asphyxia. Almost every inspiration is immediately followed by two or three cardiac contractions succeeding each other at very short intervals.

[†] It is important to notice that the convulsion of asphyxia is identical with that produced in Kussmaul and Tenner's experiment, both having the expiratory cha-

111. State of the Circulation in Asphyxia.—This may be best studied by actually observing the condition of the heart and great vessels in a narcotized animal, of which the chest has been opened while respiration is maintained artificially. In a perfectly chloralised animal, the heart may be exposed very rapidly, as follows:—The integument covering the left side of the chest having been turned back, a series of strong ligatures are passed round the costal cartilages, close to the left edge of the sternum, in such a way that each ligature enters the thoracic cavity by one intercostal space and passes out by the next; a second set of ligatures are passed in a similar manner round the ribs in a vertical line outside of the præcordia. The ligatures having been tightened, the quadrangular space between them can be cut away without any bleeding. The pericardium having then been opened, the thoracic organs can be perfectly well seen. If now after continuing the artificial respiration till apnœa is produced, it is suspended, all the degrees of respiratory activity, viz., apnœa, natural breathing, hyperpnœa, dyspnœa, convulsion, asphyxia, will be witnessed in the order in which they have been mentioned, and it will be seen that no very obvious change in the condition of the heart and great vessels will occur until the last stage (corresponding to what I have called the second stage of asphyxia) is approached. During the convulsive struggle, and particularly towards its close, the heart enlarges to something like the double of its fomer dimensions, this enlargement being due (as the attentive observer will have no difficulty in satisfying himself) to the lengthening of the diastolic interval and to the quantity of blood contained in the great veins, which in fact are so distended, that if cut into they spirt like arteries. If at this point air is again injected, the heart begins after a few seconds to contract more rapidly, and in a moment or two, emptying itself of its overcharge of blood, resumes its former size.—The effect of these changes on the arterial pressure can be best studied in a curarized animal, of which the crural or caroid has been connected with the kymograph. If in such an animal, artificial respiration is discontinued till the arterial pressure, after first increasing, sinks as low as 20 to 40 millimeters, the tracing shows

racter. If that experiment is performed in an animal in the state of apnœa, the arrest of the arterial circulation in the intra-cranial nervous centres at once induces respiratory movements; and if the closure of the arteries continues, the animal passes through the successive stages of dyspnœa, and finally becomes convulsed just as in asphyxia. If at this point the arteries are released, the animal relapses gradually, after one or two vigorous inspirations, into the condition of apnœa.

that the diastolic intervals are much lengthened. If then air is injected, the arterial pressure after an interval of 5 or 6 seconds suddenly rises, while the curve expressing the rise indicates the extreme frequency of the contractions by which the heart empties itself of its contents, or rather pumps on the blood contained in the over-full veins to the arterial system. (*See* fig. 264, in which *i* indicates the moment of injection of air.) During this effort, the mercurial column usually rises above the normal, but after it is over, subsides to a height which is nearly the same as that about which it oscillated before artificial respiration was suspended.—The explanation of these phenomena may be given in a few words. One of the effects of diminishing the proportion of oxygen in the circulating blood is to excite the vasomotor centre, and thus determine general contraction of the small arteries. The immediate consequence of this contraction is to fill the venous system, in the production of which result the contraction of the expiratory muscles of the trunk and extremities powerfully co-operates. The heart being abundantly supplied with blood, fills rapidly during diastole and contracts vigorously, in consequence of which and of the increased resistance in front, the arterial pressure rises. This last effect is however temporary; the diastolic intervals being lengthened by the excitation of the inhibitory nervous system, and the heart itself weakened by defect of oxygen, the organ soon passes into the state of diastolic relaxation already described. Its contractions become more and more ineffectual till they finally cease, leaving the arteries empty, the veins distended, its own cavities relaxed and full of blood.—That the small arteries are contracted in asphyxia we learn by inspecting them (*see* § 49). The narrowing is as marked as it is during electrical excitation of the medulla oblongata. In both cases the contraction induces increased arterial pressure, but there is this difference, that whereas in the latter case the heart is not interfered with, in the former its functional activity is much impaired by the condition of the blood. Consequently, the rise of the arterial pressure is much greater in proportion to the degree of contraction of the arteries during excitation of the medulla than in asphyxia.

112. **Examination of the Heart after Death by Asphyxia.**—If the heart is rapidly exposed immediately after death by asphyxia, and a strong ligature tightened round the roots of the great vessels, the organ may be readily cut out without allowing any blood to escape from its cavities. The quantity of blood contained in the right and left side respectively may be measured by carefully opening the ventricles and allowing their contents to flow into separate measure glasses. It is always found that all the cavities of the heart are filled to distension, the quantities

in the right and left cavities respectively, usually being to each other about in the proportion of 2 to 3. The lungs are always pale; if, however, the body is kept for a few hours, those parts of the organs which are lowest become airless and soaked with sanguinolent liquid.

113. Demonstration of the Chemical Changes which occur in the Blood in Dyspnœa and Asphyxia.—It being known that in suffocation two changes take place in the chemical condition of the blood,—diminution of oxygen and increase of carbonic acid gas,—it is obviously not unreasonable to attribute the phenomena either to the one or the other of these changes, or to the combination of both. In the preceding pages it has been assumed that they are due to the diminution of oxygen. The chief proofs that this is so are as follows:—In order to demonstrate in a striking way and in one experiment that diminution of oxygen in the air breathed does, and that excess of carbonic acid gas does not, produce the phenomena of dyspnœa, the following method, devised by Rosenthal, may be employed. The mercurial gasometer (fig. 251) is filled with oxygen. The animal is then allowed to breathe the gas in the way previously described (§ 95) until it may be reasonably supposed that the air contained in the air passages is displaced by it. This occurs in the rabbit in about ten respirations. The communication is then opened between the valve B and the receiver, while the exit tube is clipped so that the animal both inspires from the gasometer and expires into it. As the experiment goes on, it is obvious that the proportion of carbonic acid increases and must continue to increase, until that gas attains such a tension in the gasometer that no further escape from the blood is possible. At first the volume of gas in the gasometer undergoes no sensible diminution, for the animal expires nearly as much of carbonic acid as it inspires of oxygen; afterwards, as the quantity of carbonic acid gas given off becomes less and less, the cylinder sinks in each inspiration more and more. As soon as this is the case it is of course absolutely certain that the animal is breathing an atmosphere containing a large excess of carbonic acid gas, yet notwithstanding, there is no sign of asphyxia, the reason being that the oxygen still exists in the mixture in a proportion exceeding that in which it exists in the atmosphere, or at all events, not falling far short of it. When at a still later period the breathing begins to be excessive, the dyspnœa can at first be relieved by increasing the pressure to which the gases contained in the gasometer are exposed. This of course while it favours the absorption of oxygen, equally favours that of carbonic acid gas; that the latter has *no* physiological effect cannot be maintained, but the experiment proves that its effect is very inconsiderable.

The *direct proof* that dyspnœa is dependent on defect of oxygen, is obtained by the analysis of the gases of the blood in an animal which has been asphyxiated by the inhalation of pure nitrogen. Pflüger has found that an animal (dog) breathing nitrogen becomes hyperpnœic in 15 seconds. In 20 seconds the struggle is at its height, the blood being already very dark. In Pflüger's experiments, blood was allowed to flow from an artery into a recipient for the analysis of its gases, at from half a minute to a minute after the beginning of the inhalation of nitrogen, the animal being already in the second stage of asphyxia. It was found, for example,* that the blood of an animal which before breathing nitrogen contained 18·8 per cent. per vol. of oxygen (at 760 millim. and 0° C.), contained after breathing nitrogen for one minute *a mere trace of oxygen* (0·3 *per cent.*); during the same period the carbonic acid gas had diminished from 47·2 per cent. to 39·4 per cent.—These experiments are referred to here on account of their fundamental importance. They are much too difficult for repetition.

114. Demonstration that the Pulmonary Terminations of the Vagus Nerves are Excited by Distension of the Lungs. —It was long ago surmised by physiologists (particularly by Rosenthal) that the pulmonary branches of the vagus nerves contain afferent fibres, which are excited by the expansion of that organ, and that these fibres take part in the regulation both of the movements of the heart and those of respiration. The proof of this has been lately given by Hering. A dog having been narcotised with morphia or opium, one arm of a T-shaped cannula is secured in the trachea, the other being connected with a mercurial manometer. To the stem an india-rubber connector is fitted, which is guarded by a screw clip, and ends in a blowing tube: a cannula is placed in the carotid and connected with the kymograph. These preparations having been made, an observation of arterial pressure is taken. The clockwork being still in motion, the experimenter distends the lungs of the animal until the distal column of the manometer stands about 30 or 40 millimeters above the other, and then closes the clip. Two important results are produced. In the first place, the inspiratory muscles are thrown out of action and remain relaxed so long as the distension lasts, while those of expiration are brought into continuous and energetic contraction; and secondly, the frequency of the contractions of the heart is more than doubled. In the preceding experiment the circulation is considerably affected by the increased pressure exercised by the distended lungs on the heart and great veins; consequently, the increased frequency of the pulse might be attributed in whole or in part to this circumstance rather than to the pulmonary distension. To meet this objection, the experiment may be modified as follows:—A dog is narcotised and respiration maintained artificially, the apparatus being so arranged that at any moment the lungs may be distended as in the last case. This done, the thoracic organs are completely exposed by removing the anterior wall of the chest in the manner described in § 49: it is then seen that the effect of inflation on the heart is just the same as when the thorax is closed. These results are sufficient to show that pulmonary distension and acceleration of the contraction of the heart, stand in the relation to each other of cause to effect. That the influence of the former on the latter is exercised through the nervous system, and consequently through the vagi (these being the only known channel by which the lungs are in communication with the nervous centres) is sufficiently obvious. Accordingly, we should expect that if this channel were interrupted the effect would be annulled, and experiment proves that it is so. The demonstration is, however, very difficult, for in the dog the pulsations of the heart are already so rapid after section of the vagi that no further acceleration is possible; a negative result, therefore, would mean nothing. Hering has met this difficulty by carefully exciting the peripheral end of one of the divided nerves after section of both (using Helmholtz's modification), so as to reduce the frequency of the heart's action, and repeating the pulmonary distension under these altered conditions; the result was still negative. These experiments teach us two important facts relating to the innervation of the lungs, viz., that the pulmonary branches of the vagus contain afferent fibres, the excitation of which by pulmonary distension tends to weaken or paralyse both the inspiratory and cardiac centres in the medulla oblongata; the one action showing itself in the complete cessation of the rhythmical efforts of the inspiratory muscles, the other in the shortening of the diastolic intervals of the heart. The subject requires much fuller investigation than it has yet received.

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* Pflüger's Archiv., Vol. I. p. 94.

CHAPTER XVIII.

ANIMAL HEAT.

The temperature of the body is dependent on the relative activity of two sets of processes, viz: those by which heat is produced or generated, and those by which it is destroyed or lost. The subject admits of being correspondingly divided into two parts—the study of the processes, and the study of the resulting state. The former is based on the measurement of the quantity of heat set free at the surface during a given period (Calorimetry); the second on the measurement of the temperature existing in the circulating blood and the tissues at the moment of observation (Thermometry).

Section I.—Calorimetry.

The production of heat is one of the essential functions of living tissue; consequently, wherever there are living cells, heat is generated at all times. We assume at the outset that the source of production is the sum of the chemical processes which take place *in* the body; and that under all circumstances, so long as the tissues are neither growing nor wasting, the quantity of heat produced by the oxidation of the food consumed is equal to the quantity which would have been produced had the same quantity of oxidizable substance been converted into similar more or less oxidized products *out* of the body.

115. There are two distinct methods by which a theoretically complete determination of the quantity of heat products in the body in a given time can be arrived at. The first consists in *deducting* the heat-producing power (heat value) of the substances discharged from the body in a given time, from the heat value of the substances consumed. The second is based on the actual measurement of the quantity of heat discharged in a given time. In the former case the *difference* obtained expresses the amount of heat produced in the period *provided that the animal is in a state of nutritive equilibrium—i.e.*, that its tissues are neither growing nor wasting. In the latter, the measurement gives the

desired result *provided that the discharge is exactly equal to the production of heat*—*i.e.*, that the temperature of the body remains the same.

With reference to the first method, as it reposes entirely on chemical and physical operations, some of which do not fall within the scope of this work, while others will be described under other heads, all that is necessary is to make clear the principles of its application.—So long as an animal is in nutritive equilibrium (*see* above) the combustible material actually *consumed*, *i.e.*, oxidized in its body in a given time, may be known by deducting from the quantity of such material actually swallowed, the quantity discharged in the fœces. This determination is therefore purely a question of chemical analysis.

The heat-producing powers of the chief constituents of food have been determined approximatively by Frankland, who finds, for example, that one gramme of albumin, in undergoing complete combustion into water, carbonic acid and ammonia, produces heat enough to raise 4,998 grammes of water one degree centigrade. This fact we express by stating that, 4,998 is the heat value of albumin. In like manner Frankland has found the heat value of lean beef to be 5,103, and of the fat 9,069. If, therefore, it were possible to determine how much of any of these substances is consumed, say per diem, it is clear that we could readily calculate how much heat would be produced, provided that the consumption, *i.e.*, oxidation, were complete. As regards the albuminous elements of food, no such complete oxidation takes place, for the elements of these compounds do not leave the organism in the form of ultimate products of oxidation, but in great part in the form of urea and other imperfectly oxidized organic constituents of urine. The quantity of heat actually produced by a given weight of albumin, therefore, falls considerably short of its heat value. In order to arrive at this quantity, the deduction previously referred to must be made: *i.e.*, from the heat value of the albumin consumed, the heat value of the nitrogenous excreted substances into which it is transformed must be taken: the difference expresses theoretically the exact number of heat units actually generated by its elements in their passage through the body. As regards the hydro-carbons, no such deduction is necessary, so that in the case of animals which feed exclusively on these compounds—*e.g.*, bees—the quantity of heat produced is at once obtained by estimating the heat value of the food consumed.*

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* No results can be obtained by this method unless the animal is in a state of perfect nutritive equilibrium. For this reason, it can be seldom applicable in the investigation of physiological or pathological questions relating to heat; for, on account of the length of the periods over which the determinations must necessarily extend, it gives little or no information as to the *variations* in the production of

Another chemical method of estimating the rate of production of heat in the body of an animal, is founded on the estimation of the discharge of carbonic acid from the lungs and skin. In carnivorous animals this method is of little value, for, as we have seen, so much of the food consumed as consists of albuminous compounds is incompletely oxidized, so that there is no definite relation between the consumption of albuminous products and the amount of oxidation. In such animals, however, as can be fed entirely on hydro-carbons of known composition, the carbonic acid gas discharged may be taken as an exact index of the heat production—not because the quantity of heat produced, as was at first erroneously assumed, is equal to the heat which would be disengaged by the oxidation of the quantity of carbon actually contained in the carbonic acid, and of the quantity of hydrogen contained in the corresponding quantity of water—but because in such an animal the whole of the material consumed is completely oxidized; so that the quantity of carbon discharged as carbonic acid is always equal to the total quantity of the same element oxidized. On this account bees, which can be fed exclusively on hydro-carbons, and have the additional advantage that, although they are of variable temperature, their heat production is as active as that of warm-blooded animals, are specially adapted for the investigation of the relation between heat production and oxidation.

Under many circumstances which preclude the use of this method alone, it is of value in combination with that of direct measurement, to be immediately described; for the information it affords, even when the nutritive substances consumed are partly nitrogenous, is trustworthy. If the ingestion of nutritive material is regular and uniform, it affords a rough, but otherwise reliable, indication of whatever variations may occur in the activity of the chemical vital processes.

It will be readily understood, that these indications occur later than the causes which produce them; so that it is not until some time after any increase or diminution of oxidation, that the corresponding increase or diminution of the discharge of carbonic acid manifests itself. The mode of gauging the discharge of carbonic acid in the animal body has been described in the previous chapter. In the application of the results of such determinations, it must not be forgotten that the absolute values obtained are meaningless. Their use is limited to the interpretation of direct calorimetrical measurements.

116. Direct Estimation of the Quantity of Heat produced by an Animal in a given Time.—The second method (to which alone the term Calorimetry is strictly applicable), consists in the

heat, the appreciation of which is practically more important than the determination of the means of the quantities produced per hour or day.

direct estimation of the quantity of heat (heat units) given off by an animal in a given time. The subject of observation is placed for a measured period in a chamber, which is so constructed that while it is continuously supplied with air for respiration, it is surrounded on all sides by a mass of water, the weight and temperature of which are known. The construction of such a chamber (Calorimeter) can be readily understood from the diagram, fig. 265.

A, is a box of zinc plate, in which the animal is placed, the size varying according to the animal it is intended to receive. If for rabbit or small dog, it is 15½ inches long by 12 inches wide, and 13 inches high. It possesses two openings, one of which is in the lid and communicates with a large gasometer, into which air is constantly injected by a Bunsen's water air-pump. The other is in one end, and opens into an exit tube (D), which after surrounding the box twice, terminates in a flexible connector, by which the air which has passed through the chamber escapes. The section of this tube, the purpose of which is to secure the condensation of the aqueous vapour discharged from the lungs and skin, is oblong and rectangular; in order that it may present to the water by which it is surrounded as large a surface as possible. The inner box (A) is surrounded by another, which is of such dimensions that the external surface of the former is separated from the internal surface of the latter by a space of an inch and a half in every direction. This space contains water the weight of which can be readily known. The inner box can be fixed into its place by a simple mechanical arrangement. The water-chamber (B) is contained in a wooden case (C), which however is so large that a considerable space intervenes, which is closely packed with tow, the purpose of which is to prevent loss or gain of heat by radiation or conduction, and thus to render the temperature of the interior of the apparatus entirely independent of that of the surrounding media. For the same reason the external surface of the water-chamber is of bright tinplate. The interior of the water-chamber is japanned. The zinc inner chamber for the reception of the animal is left as it is.

The temperature of the animal having been measured by passing a thermometer an inch and a half into the rectum, it is placed in the box, the exit tube of which has been previously brought into communication with an aspirator. The lid is then rapidly but carefully closed with putty, and the whole placed without loss of time in the water-chamber. The water-chamber is then closed and immediately covered with a layer of tow. In its lid there are two oblong openings for the introduction of stirrers.* The water having been agitated immediately after the introduction of the box containing the animal, a thermometer is introduced by one of the openings already mentioned, which after three minutes is read. The time having been noted, the apparatus is left to itself for fifteen minutes, half an hour, or an hour, and the temperature is again observed after agitation of the water. The results having been noted, the animal is withdrawn with as little delay as possible from the case containing it, and the thermometer is introduced into the rectum to the same distance as before, and read after the same interval of time.

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* I have lately adopted a better method of agitation, consisting in the injection of air into the space below the chamber A. The construction is such that the whole of the air so used finds its way into the chamber.

In this way obviously four readings are obtained—those of the animal and of the calorimeter at the beginning and end of the given period. To interpret them we must take into account, not only the relative weights of the animal and of the calorimeter, but their several capacities for heat. In the case in which the temperature of the animal remains the same, the amount of production being equal to that of discharge, all that is required is to know how much heat has been communicated during the period of observation to the calorimeter. In the opposite case we must, in order to judge of the quantity of heat produced, add to or deduct from the quantity communicated, the quantity it has borrowed or given off from its own body. If the animal loses heat while it is in the chamber, the heat it gives off is only partially generated, the remainder being abstracted from its own body. If it gains, the quantity of heat generated is only partially given off; the remainder is added to its own temperature. To make this deduction, or addition as the case may be, two questions must be answered.

1. How much heat does the calorimeter require in order that its temperature may be raised one degree?

2. How much does the body of the animal require for the same purpose?

In both cases the quantity required is equal to the specific heat multiplied by the weight. The *mean* specific heat of the calorimeter is obtained by adding together the products of the specific heat and weight of the parts of which it is composed—*i.e.*, the iron case and the water.

Supposing, *e.g.*, the iron to weigh 3,800 grammes and the water 8,600 grammes, the specific heat of iron being 0·114, the product in question is for the iron casing 419·5, while that for the water is 8600·0. Consequently 9019·5 gramme-units* of heat are required to raise the whole one degree of temperature. Applying the same method to the animal body, the specific heat of which is estimated to be 0·83, we have of course 0·83 gramme-units as the quantity to be added or deducted for each gramme of weight and degree of variation of temperature.

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* The absolute amount of heat (in gramme-units) required to raise the calorimeter 1° C. of temperature may be ascertained empirically by introducing into the calorimeter (in place of the animal) a metal vessel containing a known weight of water at a known temperature—say 40° C.—and determining on the one hand the loss of heat sustained by the water, and on the other, the gain by the calorimeter in a given time.

The whole process will be readily understood from an example, the weight of the calorimeter being that given above.

Temperature of calorimeter—at beginning 9°·1 C., at end 9°·7 C.
„ animal „ „ 39°·2 „ 38°·3.
Weight of animal 3,200 grammes.

From these results we obtain :—

1. Units of heat communicated to the calorimeter 9019·5 × 1·6 =14431.
2. Units of heat borrowed from the body of the animal 3200 × 0·83 × 0·9=2390.

Result 14431 − 2390=12041.

That is to say, the animal, during the period of observation gave off 12,041, gramme-units of heat.

In calorimetrical experiments, the temperature of the water should, as a rule, be a little higher than that of the surrounding atmosphere. Not only is this the condition most favourable to the accuracy of the observations, but it is most advantageous as regards the state of the animal observed. If the temperature is too high the disengagement of heat from the surface is relatively lessened, so that unless completely compensated for by increased evaporation, the bodily temperature of the animal will rise. If, on the other hand, the temperature of the calorimeter is lower than that of the surrounding air, that of the animal sinks so quickly that its condition is no longer normal. It is obviously of great importance that the observations should be made in a room of even temperature, and it is desirable that it should not be too cold.

The method above described may be applied not only to the investigation of periodical and other physiological variations of the process of nutrition, but to the investigation of many abnormal states and alterations, such for example as those of fever, changes affecting the condition of the surface of the body, changes affecting the circulation, respiration or nervous system, and changes produced by the action of various drugs.*—For the investigation of fever, the pyrexial state may be produced experimentally, either by injecting into the venous system small quantities (5 to 15 minims) of the exudation liquids of certain acute inflammations; or by producing a local inflammation, *e.g.*, by applying croton oil to the surface. Although the increase of temperature produced by these methods has been carefully investigated by the thermometer, no sufficient investigations have as yet been made as to the quantity of heat produced in a given time. Among other subjects which admit of calorimetrical investigation, that of the remarkable effects produced in rabbits by the process of "varnishing" may be referred to.

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* Considering that it is not possible, even with the utmost care, to keep the animal in a perfectly natural condition during a calorimetrical observation, and that there are certain sources of error inseparable from the method itself, which do not admit of being corrected for, it is advisable in employing the calorimeter for physiological investigations to estimate the value of the results obtained not by calculation but by comparative experiments, *e.g.*, (1) by comparing the result obtained under the condition to be investigated with the result obtained in the normal state of the same animal; (2) by employing in each observation two calorimeters, in one of which the animal is placed, while the other remains unoccupied, but in all other respects under the same conditions. In this case, the loss of heat, if any, during the period of observation in the empty calorimeter is to be added to the gain in the one in which the animal is contained.

117. Increased Discharge of Heat of "Varnished" Rabbits.—It is well known that rabbits when smeared over the clipped surface with gelatin or any other similar material, die; the pulse and respiration being first accelerated and then diminishing. Associated with this last change is a very rapid loss of temperature, while the urine becomes albuminous. Formerly it was supposed that these changes were dependent on the suspension of respiration. It is easy, however, to prove experimentally that it is not so by placing the animal in a chamber at a temperature of about 30° C., when it is seen that as the temperature of the body rises the other symptoms disappear. Even if the animal has been allowed to cool as low as 21°·1 C. it can be restored by warmth. By placing a varnished rabbit in the calorimeter, it can be shown that although its temperature is actually 10° or more below that of the surrounding air, it gives off a great deal more heat than a normal rabbit. Thus I find that a rabbit which, in the normal state gives off only 3,000 heat units in ten minutes, gives off about 20,000 after varnishing, notwithstanding that in the former case its temperature was constant at 39·5° C., while in the other it sank from 36° to 25°.

SECTION II.—THERMOMETRY.

The temperature of the animal body is measured either by the mercurial thermometer or thermo-electrically:—

118. Measurement of Temperature by the Mercurial Thermometer.—The mercurial thermometer used for physiological (as well as for pathological) purposes should have the following characters. The proportion between the quantity of mercury contained in the bulb, and the lumen of the tube must be such that the difference of length of the column produced by any given increase of temperature shall be as great as possible. One degree of the centigrade scale should be sufficiently distant from another to render it possible to read easily to a tenth. On this account the range of graduation is necessarily limited. It is sufficient if it extends from 30° to 45° C. The bulb must expose a large surface in proportion to the volume of mercury it contains; for which reason it is made cylindrical. The most celebrated thermometers are those of Dr. Geissler, of Bonn. They are 32 centimeters (= 12 inches) long, and relatively exceedingly narrow —only a line and a half in diameter. The cistern is no wider than the stem, and is eight-tenths of an inch long. Mr. Hawksley, of Blenheim Street, has recently constructed for me instruments which are very similar and comparable in quality to those of Geissler. The bulbs and stems are of equal diameter throughout, not exceeding three millimeters. They are extremely sensitive, and the graduation is so fine that to the practised observer it is easy to read accurately to the 50th of a degree of Celsius.

For many purposes, it is desirable to employ maximum thermometers, *i.e.*, thermometers in which the capillary tube possesses a narrowing at one part, which, while it allows the mercury to ascend, prevents its return to the cistern; so that the instrument, when removed from the part, still shows the temperature to which the bulb has been exposed. Maximum thermometers are constantly used for clinical purposes in this country, and are also valuable to the physiologist.

If it is intended to observe the temperature in the interior of the heart, or in any of the great cavities of the body, the animal must be of large size, and must be curarized. To observe the temperature in the right ventricle, the bulb of a long stemmed thermometer must be introduced through the external jugular. To observe that of the aorta, or left ventricle, the carotid must be opened. If a large dog is used, a thermometer introduced into the right side of the heart may, if the tube be long enough, be easily pushed onwards into the vena cava. In the rabbit it is scarcely possible to do this, but it is easy with one of the thermometers mentioned above to measure the temperature of the heart in this animal.

119. Electrical Measurement of Temperature.—If a magnetic needle is set in an oblong quadrangular frame, of which one of the long sides is of bismuth and the other three sides are of copper, the two metals being soldered together at the two junctions in such a manner that the needle can swing freely in a plane at right angles to that of the frame, and so placed that the frame is in the magnetic meridian, it can then be observed that if one of the junctions is warmed, the magnet is made to decline from its normal position—the degree of deflection varying with the difference of temperature of the two junctions, and continuing until they again resume the same temperature. The deflection of the magnet indicates that in the quadrangle a current exists, and the direction of the deflection shows that the current flows from the bismuth to the copper, beginning at the warmer of the two junctions. Similar results are obtained when other combinations of two metals are substituted for bismuth and copper. According to the electro-motive force yielded by each, the metals may be arranged in what is called the thermo-electrical series; in which series those metals are placed furthest apart which yield the greatest quantity of electricity at their junctions. Bismuth is at one end, antimony at the other; close to bismuth comes German silver, and close to antimony iron. Iron and German silver yield, therefore, nearly as much electro-motive force per degree of difference of temperature as antimony and bismuth, and are much more workable. Being further apart in the thermo-electrical series than bismuth and copper, they are preferable to those metals on that ground also.

On these facts are based the electrical method of measuring temperature. Instead of the quadrangle, we give to our junctions a convenient form for introducing them into the situations at which we desire to make our measurements. Instead of the magnet, we use the instrument known as a multiplier. This consists essentially of a magnet, surrounded by numerous coils of copper wire, in which the current due to difference of temperature between the two junctions flows. We have to describe first the junctions, then the multiplier.—As one of the reasons for preferring the electrical to the ordinary method of measurement is that the measuring instrument can be introduced with exactitude into spaces which are too small for a thermometer bulb, the form usually given to the junctions is that of a needle. These needles are generally made of iron and German silver, *i.e.*, each needle consists of two wires of iron and German silver respectively, which are soldered together at and near their points, so that the junction may be completely buried in any tissue into which the needle is thrust. The two needles forming one element are connected together, metal to metal—the iron wire forming part of both, while the two German silver wires communicate each with the two ends of the coil of the multiplier, thus completing the circuit. As the needles require to be handled by the experimenter, it is necessary to protect the upper ends by covering them with silk and varnish; and the two wires must be carefully isolated from each other everywhere excepting at the points where they are soldered together.

For the purpose of making clear the mode of using the thermo-electric needles, let us suppose that it is required to measure the difference of temperature between two symmetrical parts on opposite sides of the body, one of which is inflamed, the other in the normal state. One or any number of thermo-elements may be used, each of which consists of a pair of needles with their wires arranged as above described. If only one element is employed, one of its junctions is placed in each of the tissues of which the temperature is to be investigated; the iron wire of each needle being in communication with that of the other, and the German silver wires with the ends of the multiplier. If several pairs are used, an equal number of needles must be placed in each of the parts to be compared, the arrangement of which is as follows:—Let us designate the needles on the right side A, B, C, those on the left A′, B′ and C′. The German silver ends of A and C′ being connected with the multiplier, the iron end of A is connected with that of A′, the iron end of B with that of B′, and that of C with C′, and the German silver end of A′ with that of B, and that of B′ with that of C. It is scarcely requisite to say that the junctions need not assume the form of needles; each may consist of two wires of different metals soldered together endwise, in which case it is of course necessary to transfix the part to be investigated with the joined wire, placing it in such a position that the junction is at the point to be investigated.

The multiplier consists essentially of a magnetic needle, suspended horizontally in the centre of a coil of wire along which flows the current which requires to be measured. In consequence of this arrangement, the needle, if it is in the same plane with the coil which surrounds it, will be deflected in accordance with Ampère's law, whenever a current passes along the wire, and will be acted on similarly by all parts of the coil. In order to enable the needle to act under the direction of the coil without being affected by terrestrial magnetism, it is made astatic. Two magnetic needles of equal powers, placed parallel to each other, are rigidly united by a copper wire passing through their centres of gravity in such a manner that the north pole of the one is opposite the south pole of the other, and vice versâ. The united needles are hung at such a level that the one swings above the coil, the other in its centre. From this arrangement it results not only that the influence of earth-magnetism is neutralized, but that both needles are affected in the same way by the current.

The construction of the most important parts of the instrument (which is represented in fig. 265, *bis*) is as follows:—The wire is coiled round a frame of wood, represented at *a*, the two pieces *x* and *y* are hollow. In the cavity of the horizontal piece, *x*, the lower of the two magnets swings, and can be introduced through the vertical slit in *y*. The magnets are shown at *b*. The copper wire is carefully covered with silk, and varnished. As the resistance of the coil must be low, the wire is not longer than from 20 to 25 feet, and its thickness is considerable (0·5—1 millim.).

The end of the coil terminates in the screws seen on the right side in the drawing. The needles are hung by a cocoon fibre to the centre of the frame, the mode of attachment being such that by raising or depressing the knob the height at which they are suspended can be varied. When the instrument is used, the lower needle must swing freely in the horizontal split, the upper above the graduated circle. Having raised the needles by the cocoon fibres till they swing freely, adjust the instrument with the levelling screws so that the fibre hangs exactly in the centre of the circle, then rotate the coil until the upper needle points to 180° and 0°, and connect the screws with the thermo-elements, with the intervention of a single "plug-key." If the temperatures of the junctions are different, the needle is deflected on opening the key.

In using the multiplier, it must be remembered that although the deflection of the needle varies with the intensity of the current, and consequently with the difference of temperature between the two junctions, the variations are not proportional, so that, *e.g.*, a deflection of 30° does not indicate a current twice as strong as a deflection of 15°. The relation between the readings and the intensities of the currents they indicate is different in each instrument, and consequently, must be determined once for all for each. Of the various modes which may be adopted for this purpose, the simplest is the empirical method devised originally by Melloni, an account of which will be found in all treatises on physics; the operation can be best done in a physical laboratory. Within twenty degrees the deflection is usually so nearly proportional to the strength of the current, that the error may be disregarded. For deflections beyond this point the results of the graduation must be recorded in a table of the following form, which must be kept with the instrument.

Deflection.	Intensity of Current.
20°	20·0
24°	25·0
28°	31·5
32°	39·6
36°	49·5
&c.	&c.

The above numbers are taken from the example given by Melloni. In the second column the starting number 20, stands for the intensity of current indicated by a deflection of 20°. This being assumed, the other numbers represent the intensities corresponding to the deflections opposite to which they stand.

The instrument having been graduated, it is still necessary to determine for each element the *constant*, by which the starting number must be multiplied in order to give the temperature difference. Thus, if with a certain element a deviation of 20° is produced by a difference of temperature amounting to 0·10 C., the temperature corresponding to any other deviation is obtained by multiplying the number opposite to it in the table by 0·005, which is therefore the *constant* required. This determination the physiologist must make for himself. It is effected by immersing the junctions into two large vessels containing water or oil, the difference of temperature between which is measured by accurate and sensible thermometers. To avoid error, it is of course necessary to repeat the observation many times.

[For the accurate measurements of temperature which are required in some physiological and pathological researches the multiplier is not adapted. We substitute for it a true galvanometer. The instrument used in Germany is the Spiegelbussole of Wiedemann, a description of which will be found in Rosenthal's "Electricitätslehre für Mediciner." In England, the preference is given to the galvanometer of Sir William Thomson. In both of these instruments the deviation of the needle has a definite relation to the intensity of the current, the intensities of any two currents being proportionate to the tangents of the angles of the deviation they produce; so that, so long as the same junctions are used, if the deviation produced by any known difference of temperature has been ascertained empirically, the values of other readings can be deduced from it.]

120. Distribution of Temperature in the Body.—The principal purpose to which the thermo-electrical method is applied in physiology, is that of measuring the differences of temperature which exist between different parts of the body. These differences vary according to the proximity to, or distance from, the surface of the point where the measurement is made, and according to the supply of blood which the adjacent tissues or organs receive. Taking as a standard of comparison the temperature of blood in the aorta, the facts hitherto ascertained as to the temperature of other parts are as follows:—

1. The blood of the inferior vena cava is warmer, that of the superior, colder; but in the former this is true only of the upper part of the vein just as it passes through the diaphragm.

2. The temperature of the skin and subcutaneous tissue is always considerably lower than that of the aorta, but varies a good deal.

3. The temperature of the lungs also varies. Near the diaphragm it is higher than that of the aortic blood, but elsewhere, and particularly near the costal surfaces, it is lower.

4. All the abdominal organs have a higher temperature than that of the aortic blood, those in the upper part of the abdominal cavity being the warmest.

5. The blood contained in the right ventricle is somewhat warmer than that in the left, the difference varying from 1° C. to 3° C. This difference is not dependent on the cooling of the blood as it passes through the lungs; for it is just as marked when an animal is made to breathe warmed air saturated with moisture. Moreover, such an hypothesis is rendered untenable, by the fact that the lungs themselves are scarcely cooler than the blood in the aorta. Its real cause is, doubtless, that the wall of the right ventricle is in contact with the diaphragm and abdominal organs, while the left is surrounded by lung.

The recent introduction of thermometers of extreme sensitiveness and accuracy has rendered the method less important to the physiologist than it seemed to be a few years ago. This may be illustrated by the remarkable fact, that the long controversy as to the relative temperature of the two sides of the heart has been at last set at rest, not electrically, but by the thermometer.

PHYSIOLOGY.

PART II.—FUNCTIONS OF MUSCLE AND NERVE.

By Dr. MICHAEL FOSTER.

INTRODUCTORY.

In the following part of this work, the object chiefly kept in view has been to limit the directions as much as possible to such observations and experiments as the student may be reasonably expected to perform for himself under due supervision. The ordinary phenomena of muscle and nerve are consequently dealt with at far greater length than are the properties of the central nervous system. The latter are, to say the least, but imperfectly known, the experiments on which our knowledge rests difficult and complex, and too often bringing out uncertain or even contradictory results. The former, on the other hand, may be studied with approximate exactitude; the methods of experiment and observation are becoming year by year more physical in character, and the observations themselves, fundamental in their nature and having the widest bearings in all the higher branches of physiology, may for the most part be conducted on frogs, may be repeated any number of times without difficulty or expense, and so serve usefully as a means of training students in physiological study and enquiry. The phenomena in question are so fully treated of in various text-books, that space in the following chapters has been devoted to detailed instructions as to how to proceed in the various observations rather than to complete explanations of what the observations are intended to show or prove.

Instructions concerning the various special pieces of apparatus required in this part of the subject are thrown together, for convenience sake, in the first chapter. The succeeding chapters deal with the general properties of muscle and nerve; while such observations as the student may be expected to make on the central nervous system are contained in the two last chapters.

No special chapters on the senses have been introduced, as there seemed to be no mean between the common simple observations on the one hand which are found in all the text-books and such elaborate instructions on the other as would hardly come under the scope of this work.

CHAPTER XIX.

GENERAL DIRECTIONS.

I. The Nerve-Muscle Preparation.—Having pithed a frog and destroyed both its brain and spinal cord, lay it on its belly and make an incision through the skin along the middle line of the back of the thigh, from the knee to the end of the coccyx, and carry the incision along the back about midway between the coccyx and ileum (fig. 266, line *k*, *m*, *n*). On drawing back or removing the skin, there will come into view, on the outside of the thigh, the *triceps femoris* (fig. 267 *a*), on the median side the *semi-membranosus c*, and between these the small narrow *biceps femoris b*. With the "seeker" separate gently *b* and *c*; the sciatic nerve and femoral vessels will be found running between them. Gently tear away, with the seeker, the connective tissue round the nerve, beginning near the knee (where it divides into two branches), and working upwards till the muscle *n* is reached. Be careful to touch the nerve itself as little as possible, and on no account seize it with a pair of forceps. Carefully cut through the pyriform muscle *n* and the connective tissue in which the nerve is embedded at this point, divide the iliac-coccygeal muscle *d*, right through, and follow the three nerves (which come into view when the muscle is removed, and which go to form the sciatic and other nerves) right up to the vertebral column. Cut the column across just above the last lumbar vertebra, and bisect lengthways the piece so cut off. Hold the bony fragment with the forceps, lift it up and free it from the tissues around, and then follow with the scissors the nerves right down to the knee, cutting away their various branches and removing any tissue which still may be clinging to them.

Now remove the skin from the leg; the gastrocnemius (fig. 267 *g*) will at once be recognized: cut through the tendo achillis at *f*, below the thickening at the heel. Holding the cut tendon with a pair of forceps, it will be easy, with a few strokes of the seeker, to free the muscle right up to its attachment to the end of the femur, at *h*. The branch of the sciatic nerve going to the gastrocnemius will be readily seen when the muscle is turned over, as also another branch which runs along its under surface, but which ends in other muscles. Carefully avoiding any injury to the former nerve, but disregarding the latter, cut away the whole of the tibia and fibula from the femur. Clear away, carefully avoiding the nerve, all the muscles of the thigh from the lower end of the femur so as to leave the bone tolerably bare, and cut the bone across at its lower third. There is left merely the end of the femur, to which is attached the uninjured gastrocnemius, with the whole length of the nerve from the muscle up to its entrance into the spinal canal. The muscle attached to the fragment of femur, with its prepared nerve, is represented in fig. 268. (The vertebral fragment is not shown.)

II. The Lever.—In order to study the contraction of a muscle, it is advantageous to employ a lever.

The *myographion* of Helmholtz and Pflüger is shown in fig. 269. The lever *a* moves on the fulcrum *b* and is balanced by the counterpoise *c*. At *d* is either a fine brush to write on paper, or a fine style to scratch smoked glass or paper. The rod *e* bearing the style, moves on a hinge at *f*, and also carries a counterpoise *g*. Hence the writing point describes a straight line, while the actual end of the lever itself is describing the arc of a circle. The silk thread coming from the tendon of the muscle is attached at *h*. The small pan is to receive weights for loading the muscle.

For ordinary purposes, the simple lever of Marey, shown in the lower part of fig. 270, is much more convenient for use, while at the same time the momentum of the heavy lever of the myographion is avoided. The portion next to the fulcrum is of metal, perforated or notched to receive the hooks, &c., by which the muscle is attached above, and the weight below. This is prolonged by a thin slip of wood or piece of straw, at the end of which is a fine brush, placed horizontally at an angle of about 60 degrees to the long axis of the lever, or a thin slip of gutta-percha bearing a fine needle for tracing on smoked glass or paper.

To get rid of the momentum, the weight may be replaced by a long weak spiral spring. This spring must be graduated beforehand, *i.e.*, the amount of force determined which is required to extend it to a given amount. The spiral may be replaced by a simple slip of main-spring pressing on the lever in a direction opposite to that of the movement given to it by the muscle.

III. The Moist Chamber.—In order to prevent the muscle and nerve from drying, they must be kept damp. Moistening either muscle or nerve, and especially the nerve, even with Na. Cl. ·75 p. c. is undesirable, as it tends to introduce errors. It is necessary, therefore, so to place the nerve and muscle that they may be experimented upon in an atmosphere kept uniformly damp. This is effected by means of the moist chamber (fig. 270).

This consists of a platform of hard wood or ebonite, which slides up and down, and can also be turned from side to side and clamped in any position, on an upright. Let into the platform are two or more pairs of insulated binding screws for receiving the various wires for the electrodes, as well as clamps into which the leaden electrode bearers are fixed. The upright on which the platform slides also carries above a sliding arm, with a clamp for holding the femur of the nerve-muscle preparation, and below a similar sliding arm to which the lever is fixed. The attachment of the muscle to the lever is carried through a slit in the platform. A common glass shade fitting into a rim in the platform covers everything; and when several pieces of wet blotting-paper are placed inside the cover, the atmosphere within may be kept saturated with moisture for any length of time.

IV. Nerve Chamber.—When the phenomena of electrotonus (Chap. XXVII.) are being studied, it is very desirable to have a smaller chamber than the ordinary moist chamber to work in. This may be gained by having a small glass trough, about three inches long and one broad, with a moveable top, and the glass of one of the sides replaced by a piece of india-rubber sheeting with a slit along the middle. The electrodes may be introduced through the slit at the side (the india-rubber closing on them), the nerve placed in position on the electrodes, a few morsels of wet blotting-paper inserted (so as not to touch the nerve), and the cover laid on. The nerve may thus be kept from drying for a considerable time.

V. Electrodes.—For many purposes the ends of the copper wires may be used without any special arrangement. The two wires may be kept separate, or they may be fixed at a definite distance from each other in an insulating handle of bone, wood, gutta-percha, &c. (fig. 271). It is often convenient to have the ends of wires completely covered, except just at one point in each to which the nerve may be applied (fig. 271). In this case it is also frequently an advantage to have the ends somewhat curved. Such a pair of electrodes can easily be made at once by fastening two wires, bent as desired, on either side of a slip of wood, or other non-conducting material, of the thickness required to separate the wires sufficiently, coating the whole with melted paraffin, and, when the paraffin has cooled, scraping a little away at one spot till a point of each wire is exposed. Platinum wire, or slips of platinum foil, may be advantageously substituted for the terminal portions of the copper wires.

VI. Non-Polarizable Electrodes.—In many cases, however, it will be absolutely necessary to have non-polarizable electrodes. The most convenient form is that of Du Bois Reymond, modified by Donders (fig. 272).

A glass tube *a* (about one-third inch diameter is the most convenient size) is plugged at one end by a plug *b* of china clay, worked into a firm putty with ·75 p. c. sol. of Na. Cl. A few drops of a saturated solution of sulphate of zinc *c* are carefully introduced into the tube. A slip of zinc, or piece of zinc wire *z*, thoroughly amalgamated at the tip but covered with varnish over the greater part of its length, is introduced into the tube, and so placed that the amalgamated end dips into the zinc solution as far as two or three millimetres above the clay plug. The other upper end of the wire is bent round the upper open end of the tube, and brought to the binding screw of the brass collar *d*, which is moveable up and down the outside of the tube, and can be clamped at will. The copper wire *e* is fastened in the same binding screw.

Several such electrodes of different forms should be prepared. The tube may be cut off straight at the lower end, and the clay plug brought out in the form of a cone (fig. 273 A), or in any other shape that may be desirable. It is often convenient that the end of the tube should be cut obliquely, with the clay plug not projecting at all (fig. 273 C). The end of the tube may be of the same diameter as the rest of the tube, or may be brought more or less to a point. Where the electrodes require to be applied to nerves, it is convenient to have the form fig. 273 B; the end of the tube is bent round, and the extreme point closed; near the end on the upper surface a small hole is drilled; consequently the plug *b* is only exposed at *b*′.

Electrodes of different lengths should be prepared; those required for working in the moist chamber need not be more than two inches long; otherwise, five or six inches is a convenient length.

The most convenient electrode-bearer is represented in fig. 272. The piece of leaden wire *k* ends in the brass head *h*′, which bears the two arms *f f*, each of which holds an electrode tube by means of a spring collar. The two arms move round the point *h*, and can be clamped in any position. The points of the electrodes may thus be brought near to or apart from each other, as may be desired. The extremely flexible but non-elastic leaden wire (a cylindrical wire being far better than a flat piece of lead), the far end of which is fixed in a clamp, permits the pair of electrodes to be placed without rebound, and therefore with great accuracy, in any position whatever.

VII. Commutator.—This is useful for changing the direction of a current when the effects of constant currents are being studied. Any form of commutator may be used, provided that the current can easily be cut off altogether, as well as reversed in direction. A very convenient form is that represented in fig. 297, in which, when the handle is horizontal, the current is cut off from the electrodes altogether; and a different direction given to the current according as the handle is raised or lowered. The wires from the battery should be brought to the upper, and those from the electrodes to the lower binding screws.

VIII. Rheochord.—This instrument is directed to be used in the following pages simply for the purpose of causing definite changes in the amount of a constant current under use. Fig. 298 represents a convenient form of the rheochord of Du Bois Reymond.

Bring the wires from the battery to the binding screws at the top of the board and those from the electrodes to the same screws. If all the plugs are in place and the travelling mercury cups close up to the top of the board in direct contact with the brass, the resistance to the current from the battery offered by the rheochord compared with that offered by the circuit of the electrodes is practically nil, and consequently all the current passes through the former and none through the latter. If the mercury cups be moved on their platinum wires a little distance down the board, there will be no passage for the current from one side of the rheochord to the other except through such a length of the two platinum wires as lies between the cups and the brass plate. But these thin wires offer a certain resistance to the passage of the current, and consequently a proportionate fraction of the total current from the battery is thrown into the circuit of the electrodes. By sliding the mercury cups various distances down the graduated board, small differences of resistance in the rheochord are established, and consequently slightly differing fractions of the total current thrown into the circuit of the electrodes. If one of the plugs be removed, a certain amount of resistance is suddenly introduced into the rheochord, and consequently a certain amount of the current is suddenly thrown into the circuit of the electrodes. With the different plugs different amounts of resistance are introduced into the rheochord, and different amounts of the current thrown into the circuit of the electrodes. The several plugs are all numbered as multiples of the resistance offered by the total length of the platinum wires on which the cups travel. Thus if the resistance offered by the rheochord when the cups are quite at the bottom of the board, but all the plugs in place, be taken as the unit, the removal of the plug marked 5 will suddenly introduce *in addition* five times that amount of resistance, and so send a proportionate amount of the current through the circuit of the electrodes.

IX. The Double Key or Wippe.—This is very convenient when it is desired to throw a given current from one pair of electrodes into another. It is represented in fig. 299, in which it is seen that the mercury cups belonging to the binding screws 1 and 2 are connected by a handle of which the central part is of insulating material, the ends of thick copper wire. Each of the copper wires is crossed just as it enters the handle by an arch of the same material; one end of each arch dips into one of the mercury cups 3 and 4, when the handle is thrown to the right as in the figure. The wires from one pair of electrodes are to be brought to the binding screws 3 and 4, those from the other to the screws 5 and 6. The small cups on the surface are to be filled with mercury, and the wires from the battery or induction coil, &c., brought to the screws 1 and 2, and the straight cross wires between 3 and 6 and 4 and 5 taken away. By throwing the handle to the right, the current from the battery is sent into the wires connected with the screws 3 and 4; by throwing the handle to the left, into the wire connected with the screws 5 and 6.

X. The Marking Lever.—This is a two-armed, flat, metal lever, fig. 275 *a a′*, working vertically on the pivot *b*, the arm *a* being considerably heavier than *a′*. The pivot is electrically continuous with the small brass pillar *c*, where binding screws receive a wire or wires from a battery, induction coil, electrode, &c. The pillar *c* is placed on one side of the support *d*, made of non-conducting material, which by *e* can be clamped to any stand, either vertically or horizontally. On the other side of the support is a similar pillar *f* (also bearing binding-screws), on which the arm *a* of the lever rests; *g* is a weak spring, which serves to catch the end of the lever when thrown up; *h* is a slip of gutta-percha or india-rubber at the end of the lever bearing the marking needle or pen.

If *c* be connected with one of the poles of the battery, and *f* with one end of the primary coil, when the lever is down and horizontal, the arm *a* being in close contact with the pillar, the current passes along the lever from *c* to *f*. When, therefore, the arm *a* of the lever is suddenly tilted up, which can easily be done with the point of the finger, the current is suddenly broken; and the moment of the breaking is indicated on the registering surface by the descent of the marking point of the lever. When it is desired to mark the making rather than the breaking of a current, the two positive (or negative) wires must be brought to the binding screws of *f*, and the two negative (or positive) to those of *c*. The tilting up of *a* will then correspond to the making of a current as in Du Bois Reymond's key. (*See* below.)

XI. Arrangement of Apparatus for Experiment.—The electrodes being charged and fastened in their bearers, the bearers secured in the clamps, and the wires from the electrodes brought to the binding-screws of the platform of the moist-chamber, fasten the femur of the nerve-muscle preparation securely in the clamp, in such a way that the muscle hangs vertically downwards over the slit. Seize the vertebral fragment with the forceps, and gently lodge the nerves on the electrodes. Firmly fasten the tendo Achillis in a Kronecker's clamp (fig. 274), and with a strong silk thread of appropriate length connect the Kronecker with the lever, the silk passing through the slit. Adjust the arm bearing the muscle, and that bearing the lever, in such a way that the muscle hangs perfectly vertical, and, without being actually on the stretch, is so attached to the lever (which should be in a perfectly horizontal position) that the slightest contraction of the former will move the latter.

Bring the wires from the battery, induction coil, &c., to the binding-screws which carry the wires of the electrodes. Place the glass shade over the platform, with wet blotting-paper inside, being careful that the wet paper touches neither the nerve-muscle nor the wires.

Prepare weights for loading the lever; the most convenient are 5, 10, 20, 30, 50, 150, 200, and 300 grammes. Place the whole apparatus so that the point of the lever touches the registering surface.

Where it is required to study the muscle without removing it from the circulation, another method must be adopted, which may be followed in other cases as well. The upper surface of the platform should in this case be provided with a thick layer of cork.

Having pithed the frog, pin it firmly, belly downwards, on the cork of the platform, fixing the thigh of one side especially tight; one of the pins should be passed close to the femur, on the anterior surface of the thigh, just above the knee. Make a slight longitudinal slit along the tendo Achillis, which divide low down, and carefully dissect out for a few millimetres; put a small but strong S hook, with a silk thread attached, in the tendon, pass the silk over one of the small double-cone pulleys which are fixed on to the platform, and so through the arms of the T slit to the lever below.

The sciatic nerve may now be dissected out in the thigh without injuring the blood-vessels, and the curved electrodes slipped beneath it.

Where the recording cylinder used rotates on its horizontal, and not on its vertical, axis, the simple lever must be fixed in a horizontal position at the front of the platform, and the silk brought in a straight line to it from the tendon. Resistance to the action of the muscle may then be gained by means of the light spring, or a weight passing over another pulley. As a rule, it is best, when possible, to do without a pulley.

XII. Recording Tuning-Fork.—For measuring small intervals of time in physiological observations, it becomes absolutely necessary to make use of tuning-forks of known rates of vibration. Fig. 277

is a figure of a tuning-fork arranged by König for recording its vibrations on a revolving surface. A massive stand bears the fork A firmly secured in it. The two coils *c c′* (which by means of the arrangement *k* can be slid up and down the stand, so as to accommodate themselves to tuning-forks of different lengths) project over the two ends of the fork, and each bears a screw *d* which can be screwed as desired up to or away from the ends of the fork. The upper arm of the fork bears at its end a rod *a* with platinum point which dips into the mercury cup *b*.

The tuning-fork B, which must have the same rate of vibration as A, is fixed into a light moveable stand, so that it can be placed in such a position that the light elastic marker *g* may touch with the least excess of friction the recording surface. This fork is placed in the same manner as A, with its ends between the coils *e e′*, bearing similarly the screws *f f′*.

One wire from a battery *w* is connected with a binding screw at the handle of the tuning-fork A, and is thus in electric continuity with the rod *a*. The mercury cup *b* is connected by a wire *z* with the coil *e* of the fork B. The other pole of the battery is connected by *x* with the coils *c c′* of A, and thence by *y* with the coil *e′* of B.

The screws *d d, f f,′* being brought rather near to their respective forks, place a small quantity of mercury covered by a little spirit in the cup *b*, and having set the fork A vibrating a little, screw the rod *a* up or down until magnetic interruption is fairly established. B will then be found vibrating synchronously with A, and the point *g* will be found tracing curves on the recording surface, the interval of time corresponding to each curve being determined by the pitch of the fork. Screw the *d d, f f,′* as far away from their respective forks as can be done without stopping the current altogether.

XIII. Arrangement of Electrical Apparatus.—Constant Current.—Place the nerve (or muscle, when muscle alone is the subject of experiment) on the electrodes, taking care that the nerve is actually in contact with each electrode. When the non-polarizable electrodes are used, their plugs must be kept damp with the normal saline solution: avoid making them too wet, and especially do not let a bridge of fluid form along the nerve between the two electrodes.

Bring the wire from each electrode to the outer binding screw on each side of a Du Bois Reymond's key (fig. 300). Bring the wires from the battery to the inner screws of the same key. Let the positive wire, the wire connected with the copper, carbon, platinum, &c., of the battery be coloured of some definite colour, *e.g.*, red; let the wire fastened to the same side of the key have the same colour. The electrode connected with this wire will be the positive electrode, or *anode*. Let the two other wires connecting the zinc of the battery with the key, and that side of the key with the other electrode which therefore becomes the negative electrode or *kathode*, be coloured of some other colour, *e.g.*, blue.

When the key is down, the brass plate offers such little resistance to the passage of the current, compared with that offered by the nerve, &c., that the whole current will pass through the bridge of the key, and none through the nerve.

Consequently, opening the key is equivalent to throwing a current into the nerve; shutting the key, to removing the current from the nerve; during the whole time that the key is open, the nerve, &c., is exposed to the action of the current.

When the kathode (negative pole) is placed at a point on the nerve nearer the muscle than the anode (positive pole), the current is said to be *descending*; when the anode is the nearer, the current is said to be *ascending*.

Single Induction Shock. — Connect each wire from the battery (fig. 276 B), a key *b* intervening, with one of the two screws on the top of the primary coil C. Connect the secondary coil D with the electrodes E E′, a key *a* intervening.

Whenever the key *b* is opened, and the current from the battery allowed to pass through the primary coil, a current is *induced* for the instant in the secondary coil; another current is similarly induced in the secondary coil when the same key is shut; but in the interval there is no current produced in the secondary coil provided that the current in the primary coil be constant.

If the key *a* is kept open while the key *b* is being opened or shut, at each opening or shutting of *b* a single "induction shock" is sent through the nerve.

If *a* be kept open when *b* is opened, *i.e.*, when the current is allowed to pass into the primary coil (when the current is *made*), but closed before *b* is closed again, a "making or closing induction shock" only will be sent through the nerve.

If the key *a* be kept closed while *b* is opened, and opened before *b* is shut (and the current in the primary coil is *broken*), a "breaking or opening induction shock" only is passed through the nerve.

In determining the direction of the induction shock, it must be remembered that, at *making* the current in the primary coil, the current induced in the secondary coil is *opposite* in direction to that of the primary, but that on breaking it, it is in the *same* direction.

Interrupted Current.—For ordinary tetanizing purposes, the magnetic interruptor of Du Bois Reymond's apparatus is used. (*See* fig. 293.) Connect the end of the positive wire of the battery with the brass column *g*, and the negative wire with *a*;

the current then enters by *g*, passes along the German-silver spring, which when not in action is in contact by a little plate of platinum soldered on to its upper surface, with the platinum point of the screw *f*. From *f* the current passes through the brass block *e*, with its binding screw *d*, to the primary coil *c*; after traversing it, it reaches the electro-magnet *b*, and then returns to the battery through the binding screw *a*. The anchor *h*, is supported over the electro-magnet by the end of the German-silver spring; the moment that the current passes through *b*, the anchor with the spring is drawn down so as to break the current at *f*. Thereupon, the magnet ceasing to act, allows the spring to return to its former position. By sliding the secondary coil to a greater or less distance from the primary, the strength of the induced current can be varied at will.

When it is specially important to avoid unipolar action, the apparatus must be modified in the manner recommended by Helmholtz. With this view, connect the column *g* with the binding screw *d* by a side wire also marked *g*, and heighten the tip of the column *a* by means of the milled rim. This arrangement is shown in fig. 294. The current enters as before, but in its course to the primary coil it passes partly through *f* and partly through the side wire *g*. When the anchor is drawn down, as seen in the figure, the spring rests upon the summit of *a*, so that the current passes directly back, as indicated by the arrow, to the battery. The moment this is the case, the current through the electro-magnet becomes so feeble that it is insufficient to keep down the anchor, the spring rising again comes into contact with *f*, and so on. The modification of effect is as follows:—1. The induced currents are weaker, for the variations of the strength of the current are less. 2. The intensity of the opening induction current, which in the ordinary arrangement is much greater than that of the closing, is reduced so that the two are nearly equal.

If it is desired to allow the current from the battery to traverse the primary coil without passing the interruptor, so as *e.g.*, to use the apparatus for producing single opening or closing induction shocks, connect the positive wire of the battery with *e*, and the negative, as before, with *a*.

It is often advisable to use a key both between the battery and the induction coil, and between the secondary coil and the electrodes.

Current with Definite Interruptions by Means of the Metronome.—Arrange as for a single induction shock, except that, in place of the key *b*, insert the electrical metronome, an instrument which may be described as a key which is opened and closed by clock-work at regular intervals of time, the length of interval being varied at will. The key *a* may be dispensed with, as unless a special provision be made, the shocks given will be both opening and closing.

Current interrupted by Means of an Oscillating Rod.—Bring one wire straight from the battery to the primary coil, connect the other wire with a slip of thin elastic steel (the length will be determined by the rapidity of interruption required), one end of which is made fast, while at the other a needle, at right angles to, but in electrical continuity with, the steel slip, hangs over a mercury cup at such a distance, that when the steel slip is at rest, the needle is quite clear of the mercury, but that when the slip is made to oscillate, with each oscillation the needle dips in and out of the mercury. Connect the mercury of the cup with the other binding screw of the primary coil. At each oscillation of the slip, the current will accordingly be made and broken.

CHAPTER XX.

GENERAL PROPERTIES OF MUSCLE AT REST.

1. **Elasticity.**—*Obs.* I. Take a gastrocnemius, prepared as directed in Chap. XIX., sec. 1, except that the nerve may be neglected wholly; fasten the femur in the clamp of the moist chamber, and attach the muscle to the lever, as directed in sec. XI. Let the lever be perfectly horizontal.

Draw on the recording surface a straight line, on which make a mark for zero, and mark off abscissæ in the proportion of 10, 30, 50, 100, 150, 200, 300, 400, &c.

Disregarding the weight of the lever (or of the pan, &c., when Helmholtz's arrangement is used), the muscle may be supposed to have its natural length when no weight is brought to bear upon it. This may be indicated by bringing the recording point of the lever to touch the zero point on the recording surface. Next shift the recording surface until the point of the lever touches the point corresponding to 10. Then place 10 grammes in the pan, or hang a 10 gramme weight on the lever. The point of the lever will move downwards, describing a line of a certain length. This indicates the amount of extension which the muscle has suffered in consequence of being loaded with the 10 gramme weight.

Remove the weight carefully; the point of the lever will return to the point where it was before the weight was applied.

The distance of the point of attachment of the muscle and that of the point of the lever from the fulcrum being known, the actual extension of the muscle with 10 grammes may be calculated from the length of the line marked on the cylinder.

"*Muscle possesses very little elasticity* (i.e., *is very extensible*); *but that little is very perfect*; i.e., *on withdrawal of the extending force, the muscle returns very rapidly and completely to its previous length.*"

Obs. II. Now move the recording surface till the lever point stands at the mark 30; load the pan with 30, and proceed as before. Repeat at 50, 100, 200, &c. Before trying the heavier weights (300, 400), see that everything is secure, especially the clamps on the femur and on the tendon. As a general rule, the attachment of the muscle to the femur at last gives way.

With the heavier weights it will be found that the muscle returns after extra extension and upon removal of the weights towards its former length, at first very quickly, and then more and more slowly—and that even after waiting for some minutes, it does not regain its former length completely.

This falling short of a complete return is due to exhaustion (commencing death, *see Obs.* IV.). The student had better, in one set of observations, start in each case from the point of the ordinate to which the lever had returned after the previous extension, but of course from the next point on the abscissa, and in another set bring down the recording surface in each case so that the lever may start afresh from the abscissa line. The lever should be horizontal at the beginning of each trial. The pan or weight should also be allowed to descend very gradually and slowly, to avoid momentum. Where there is no arrangement for keeping the recording point in a straight line, a horizontal line drawn through the end of each curve will cut off from a vertical line, drawn through the starting-point, a line equal to the vertical distance traversed by the lever point.

If now the lines so obtained be examined, it will be found that though with the greater weights there is greater extension, yet the increase of extension caused by increase of weight gets less and less. *The extension increases not in direct proportion to the weight, but with continually diminishing increments.* If a line be drawn through the points which in each case mark the limit of extension, that line will not be a straight line as it would be if the extension were in direct proportion to the weight, but will be a curve, sinking very rapidly at first, but afterwards more and more slowly, and so continually tending to run parallel to the abscissa line; in fact, it will be an *hyperbola.*

Obs. III. Neither of the above set of observations is quite correct: to eliminate the effects of exhaustion, the observations should be repeated on the muscle within the body (*see* Chap. XIX., sec. XI.), and time allowed between each observation for the muscle completely to recover itself.

Obs. IV. Kill the muscle (either the same or a fresh one) by immersing it in water at 40° C. for five minutes.

Repeat *Obs.* I. and II. on the muscle so killed. It will be found that there is far less extension of the muscle, which, after the load has been removed, does not return to its original length.

The dead muscle, as compared with the living one, is more elastic, i.e., *is less extensible; but its elasticity is very imperfect,* i.e., *the original length is not regained.*

II. Reaction.—*Obs.* V. Having pithed a frog, place a cannule in the aorta, slit open the right auricle, and drive all the blood out by injecting the normal saline solution, *which should be perfectly neutral.* Dissect out the gastrocnemius of one side with clean instruments, and with a very clean knife cut it across through the middle of its belly. Take two slips of litmus paper, one faintly red, the other faintly blue; press the cut end of one-half of the muscle on one piece, and the other on the other. On the red litmus paper will be left a distinct blue mark where the muscle was pressed; on the blue litmus paper there will be no mark at all, or, if any, a change in the direction of red, which is distinctly less red than the blue mark on the red litmus is blue.

The reaction of living muscle, freed as much as possible from blood, is faintly alkaline.

Obs. VI. Kill the corresponding muscle in the other leg by immersion in water at 40° C. Test as in *Obs.* V. The blue litmus paper will be marked most distinctly red; the red not altered. For this a much stronger blue paper may be used. The reaction is permanent, and therefore is not due to carbonic acid.

Muscle, in dying, on entering into RIGOR MORTIS, *becomes distinctly acid.*

Obs. VII. Keep any of the above rigid muscles covered in a damp warm place. Test the reaction from time to time. The acid reaction gives way to an alkaline one, which increases rapidly in intensity, and soon far exceeds the natural alkaline reaction. This secondary alkalinity arises from decomposition.

At the same time the muscle will be found to have become very extensible, with scarcely any elasticity.

Obs. VIII. Divide a fresh muscle in two. Immerse for a few minutes one-half (A) in water at 40° C.; the other (B) in boiling water. Test the reaction of both.

A will be acid, from development of rigor morits.

B will be alkaline. Before rigor mortis had time to set in, the albumin of the muscle was coagulated. This coagulation set free a quantity of alkali (*see* Chap. XXXV.); hence its reaction.

III. *Transparency.*—*Obs.* IX. Take from a frog a portion of any one of its thin flat muscles. The *mylohyoid* is the most convenient, but the *sartorius* fig. 278 *s.*), or any other thin muscle, will do as well. The muscle must be perfectly fresh and irritable, and care must be taken that at least the middle portion of muscle is not in the least injured. Place the muscle in normal saline solution, or serum, on the unheated "warm stage," and examine with a quarter-inch object-glass.

Focus down through the middle (least injured) portion of the muscle, upon some object (blood-vessel, &c.) underneath the fibres. The distinctness with which this object is seen will be a measure of the transparency of the muscular tissue.

Keeping the eye fixed upon the above-mentioned object, heat the stage. It will be found that when the temperature of the muscle has risen to 40° C. (or a little below), the object is no longer so distinct as before, or has even become totally invisible.

On entering into rigor mortis, the muscular fibre becomes opaque.

Living muscle is very extensible, with perfect elasticity, of alkaline reaction, and considerable transparency. On entering into rigor mortis it loses its extensibility, its elasticity becomes imperfect, its reaction acid, and its transparency gives place to opacity.

CHAPTER XXI.

PRELIMINARY OBSERVATIONS ON THE STIMULATION OF NERVE AND MUSCLE.

I. Electrical Stimulation.—*Obs.* I. Get ready a nerve-muscle preparation and place the nerve on a pair of common electrodes; or simply lay bare the sciatic nerve, slip the electrodes underneath, and watch the leg for any movements indicating muscular contractions. Connect the electrodes with a battery of one, two, or three cells, a key intervening. Open the key, and after a few seconds shut it again; this is equivalent to making and then breaking a current *in the nerve*. It will be found that either at the breaking or at the making, or at both making and breaking of the current, a *single muscular contraction* is produced; but that during the passage of the current (provided the intensity be uniform) there is no contraction at all.

Obs. II. Instead of a constant current, employ a single induction shock. Each application of a single induction shock (if strong enough), whether it be an opening shock or a closing shock, will produce a *single muscular contraction*.

Obs. III. Instead of a single induction shock, employ a series of shocks rapidly following each other. These produce a *continued contraction*, a *tetanus*, which lasts during the whole time of the application of the currents, or until the muscle is completely exhausted. For this purpose use the apparatus of Du Bois Reymond.

Obs. IV. Lay bare the gastrocnemius or any other muscle, apply the electrodes directly to the muscle instead of to the nerve, and repeat the above observations. The results will be the same.

II. Mechanical Stimulation.—*Obs.* V. Pinch the nerve sharply with a pair of forceps, prick the muscle with a needle; in either case a contraction will take place.

III. Thermal Stimulation.—*Obs.* VI. Touch lightly either nerve or muscle with a hot needle; a contraction will follow.

IV. Chemical Stimulation.—*Obs.* VII. Dip the end of the nerve into a strong solution of common salt; after a little while a series of contractions running into tetanus will be observed in the muscles supplied by the nerve.

Obs. VIII. Kill the muscle and nerve by immersion for a few minutes in water at 40°. The above stimuli applied to either muscle or nerve will produce no contraction.

CHAPTER XXII.

PHENOMENA AND LAWS OF MUSCULAR CONTRACTION.

I. The Muscle Curve.—In order to study the muscle curve, the recording surface must travel with sufficient rapidity. (The chief features of the curve may be seen when Sécretan's cylinder with Foucault's regulator revolves on the second axis.)

Obs. I. Arrange a muscle preparation in the moist chamber. The electrodes should be placed at some little distance from each other on the muscle itself; the nerve consequently need not be prepared. Load with 10 or 15 grms. Underneath the point of the lever bring the recording tuning-fork to bear on the cylinder.

Arrange for a single opening induction shock, but instead of the key *b*, (Chap. XIX., sec. XIII) insert the marking key, simply introducing it into one wire from the battery, so that when the lever is down the current passes, but when it is raised (and the point depressed) the current is broken (Chap. XIX., sec. X.). The point of the marking key must be brought close under the recording point of the lever but above the recording point of the tuning-fork. Place all three recording points very carefully in the same vertical line.

The marking key being closed, and the tuning-fork vibrating, open the key *a*, and remove the break from the governor of the clock-work; when the cylinder is approaching the end of the first revolution, open the marking key, and as soon as possible afterwards stop the cylinder.

On the cylinder there will now be seen three lines of marking (*see* fig. 279); *a* is the line of the marking key, and the point where it descends indicates the moment at which the current broke into the muscle; *b* is the line of the tuning-fork, and each complete curve denotes a certain fraction of a second (determined by the pitch of the tuning-fork): *c* is the line of the muscle-lever, m^1 marks the moment of the beginning of the contraction, m^2 the curve's highest point, m^3 its termination. Draw a straight vertical line m through the point where the line *a* descends, and similar vertical (parallel) lines m, m^1, m^2, m^3, cutting *a* *b* and *c*.

m—m^1 will give by measurement off *b* the duration of the latent period m^1—m^2, of the rise; m^2—m^3, of the fall; and m^1—m^3, of the total contraction.

The rapidity of Sécretan's second axis is hardly sufficient to bring out the latent period with sufficient distinctness; but the other characters of the curve may be very well shewn.

The third, swiftest, axis may be used, but there are difficulties in managing it. Care must be taken to reduce the friction of the various recording points to a minimum; and the observation should not be taken till towards the end of the second revolution. Before that, the cylinder is far from reaching its maximum (uniform) speed; after that, the overlapping curves of the tuning-fork are difficult to decipher.

Better results are obtained if the cylinder be used horizontally (the natural position of the apparatus) instead of vertically. The lever tuning-fork and marking key will of course have to be arranged accordingly.

When the heavier myographion lever is employed, the effect of the inertia of the lever will make itself manifest in a secondary curve, at the end of the muscle curve.

(For more exact observations than are furnished by Foucault's second axis, it is better to employ the *pendulum myographion, see* Wundt Mechanik der Nerven, p. 6.)

A muscular contraction, even when produced by an instantaneous electric shock, takes a measurable time for its complete development. The contraction does not begin at the moment when the stimulus breaks into the muscle, but is preceded by a latent period. The contraction curve rises at first very rapidly, but afterwards more slowly, and having reached a maximum, declines at first slowly, afterwards more rapidly, and lastly more slowly again.

The advanced student may determine by the same method the variations in the character of the muscle curve, caused by—

1. **Exhaustion.**—*Obs.* II. Having determined with a single induction shock the natural curve, exhaust the muscle by prolonged or repeated stimulation with the interrupted current, and then repeat again with the same single induction shock as before. The curve will be not only of less height, but will be longer, *i.e.* the contraction will be slower, and the latent period especially will be prolonged.

2. **Heat and Cold.**—*Obs.* III. The temperature of the chamber may be raised or lowered by introducing a current of moist hot air or pieces of ice into it.

It is more convenient, however, to use the frog in a horizontal position, simply laying bare the gastrocnemius, and dividing its tendon (*see* Chap. XIX., sec. XI.), and then placing the muscle in a double trough, made by bending a piece of leaden tube. Having determined the natural curve, pass hot or ice-cold water through the tube, and determine the curve at various temperatures.

At higher temperatures than the normal, the muscle curve is much shortened; at lower, lengthened.

3. Poisons; Veratrin, &c.—*Obs.* IV. Arrange the frog as directed for observations on muscles in the living body (Chap. XIX., sec. XI.), and having determined the natural muscle curve, inject a small quantity of veratrin ($\frac{1}{20}$—$\frac{1}{30}$ mgrm.) beneath the skin of the back, having previously divided the sciatic nerve near the knee without injury to the blood vessels. Determine the curve at given intervals after the introduction of the poison; the duration of the contraction will be enormously prolonged.

II. The Contraction as a Function of the Stimulus.

Obs. V. Arrange the nerve-muscle preparation in the moist chamber; place the nerve over a pair of electrodes. Load the muscle with about 10 grammes. Arrange for a single induction shock, using in the same series of observations the same either opening or closing shock. Draw an abscissa line on the recording surface.

Slide the secondary coil as far away as the sliding board will allow from the primary coil. Send a shock through the nerve. If there is no contraction, and most probably there will be none, move the secondary coil some centimetres nearer the primary; repeat the shock. Advance in this way, gradually bringing the secondary coil nearer and nearer to the primary, until the first visible contraction is gained.

By sliding the secondary coil backwards and forwards, accurately determine this "minimum stimulus" for the muscle and nerve under the circumstances of the case.

Advance now steadily on, moving the secondary coil a definite distance nearer the primary each time, and record each contraction as an ordinate on the abscissa line, at distances proportionate to the distances the secondary coil is moved, in a manner similar to Chap. XX., Obs. II.

The contractions will go on for a while increasing as the strength of the current increases; but at last it will be found that increasing the stimulus no longer increases the contraction, *i.e.*, the "maximum contraction" for the muscle and nerve under the circumstances has been reached. Determine accurately the relative positions of the two coils at which this point is reached.

If with the battery employed to start with, the maximum contraction is not reached, increase the number of cells.

The student in making the above observations is nearly sure to meet with very great irregularities, which will tend very much to confuse the results. These may be partly due to imperfections in the apparatus. He will therefore carefully examine these, and see that everything is in order, and especially that the battery is working steadily.

But the variations will in most cases be due to the fact that the nerve after stimulation, and the muscle after stimulation and contraction, are for a variable period of time in a different condition from what they were before. They are suffering from more or less exhaustion, re-action, &c. To eliminate these entirely is a task of considerable difficulty. They may be more or less reduced by waiting a sufficiently long time between each two trials, by working backwards from the stronger shocks to the weaker, as well as from the weaker to the stronger, &c. &c.

The student, however, will see sufficient to enable him to state that *the amount of contraction does increase with the increase of the strength of the shock (increase of stimulus), at first rapidly, then more and more slowly, and finally, when the maximum is reached, ceasing to increase any more.*

III. The Contraction as a Function of the Resistance.

Obs. VI. Everything being arranged as in the last observation, place the secondary coil in such a position as to give a shock about midway between the maximum and minimum stimulus.

First let there be no load to the muscle; record the contraction as an ordinate on the abscissa line. Then load successively with 10, 30, 50, 100, &c., &c., grammes; recording the several contractions at proportionate distances along the abscissa line.

Repeat with a minimum stimulus and also with a maximum stimulus.

With the same stimulus the amount of contraction decreases as the load is increased; but not regularly. At first, as the load is increased from zero upwards by small increments, the contraction *increases;* as the load continues to be increased, the increment diminishes, and finally gives place to a decrement. The initial increase of contraction is most prominent when the stimulus lies within a certain range of intensity.

IV. The Work Done.

Obs. VII. The dimensions of the lever being known, determine from the ordinates of contraction the actual shortening of the muscle itself during each contraction. This multiplied into the weight will give the *work done* in each case.

Draw an abscissa line and mark off from it distances proportionate to the loads employed in *Obs.* VI. Draw as ordinates the actual work done in the case of each load. A line drawn through the summits of the ordinates will give the curve of the *work done* with the same stimulus and increasing loads.

With the same given stimulus and an increasing load, the work done increases up to a maximum, and then declines.

The maximum is not the same with all intensities of stimulus.

There is a definite relation of load, muscle, and stimulus, by which the greatest amount of work can be got out of a given muscle.

CHAPTER XXIII.

THE WAVE OF MUSCULAR CONTRACTION.

Obs. I. Place a nerve-muscle preparation in a horizontal position, so that the gastrocnemius rests on some flat surface (*e.g.*, a glass plate) over which it can glide freely; clamp the femur fragment tight; by means of a pulley attach the tendon to a lever, &c., with a load of 10 or 15 grammes. Bring over the middle of the muscle the button of a light cardiograph connected with a Marey's tambour (*see* p. 254, fig. 230). If the button is large, attach to its under surface a conical piece of cork or some other material, which can be brought into contact with a small portion of the surface of the muscle.

Bring the recording point of the tambour lever to mark on the cylinder, a little distance below the other lever.

Place the nerve on the electrodes of an induction coil.

While the cylinder (first or second axis) is revolving, and the two levers are describing parallel lines, send induction shocks of various strengths through the electrodes.

The direct lever will indicate the shortening of the muscle, the tambour lever its thickening. It will be seen that they both take place at about the same time, and that with the various strengths of current the movement of one lever increases or decreases with the other.

Obs. II. Poison a frog completely with urari, so as to eliminate as much as possible the influence of nerves. Dissect out carefully one of the large muscles of the thigh; for instance, the rectus internus major (fig. 278, *r. i*). Cut away with it the piece of the pelvis, to which its origin is attached. Leave as much of the tendon of insertion as possible.

Lay the muscle in a small trough (fig. 280) (one can easily be made of gutta-percha), and place over it, as far apart as possible, two levers. The levers must be so arranged that their points write on the cylinder one below the other in exactly the same vertical line. Fix the one end of the muscle by clamping the piece of pelvis, and attach by means of a pulley a load of 5 or 10 grammes to the tendon.

Bring two pointed electrodes from an induction coil, to one end of the muscle, so that they touch the muscular fibres close to the end.

Bring the levers to trace on the cylinder rotating on its swiftest axis. While the two points of the lever are describing two parallel lines on the cylinder, send a single induction shock through the lever.

Each of the two levers will describe a curve, each curve indicating the thickening of the muscle under the lever during the contraction. But these curves will not be exactly one under the other; one, viz., that described by the lever nearer the electrodes, will be a little earlier than the other. The difference in time between the commencement of the two curves will be more marked in an exhausted muscle, or in a muscle exposed to a low temperature, than in a fresh and very irritable muscle.

The contraction then does not take place in the whole fibre at the same time, but travels with a certain velocity from the point at which the electrodes are placed along the fibre.

Obs. III. Repeat the observation; placing the electrodes on the muscle close to the tendon of insertion instead of close to the origin.

The results are just the same; the wave of contraction travels in either direction.

Obs. IV. Instead of resting the levers on the muscle as directed above, the muscle may be placed on a piece of cork with holes in it and two slips of thin foil looped round two distant parts of the muscle, each slip being connected with a lever below, as in fig. 281.

If the tuning-fork be brought to trace on the cylinder below the levers, the interval of time between the commencements of the two contractions may be exactly determined, and the distance between the two levers on the muscle being accurately measured, the velocity of the wave of contraction may be calculated.

CHAPTER XXIV.

TETANUS.

I. The Curve of Tetanus. *Obs.* I.—Having arranged the nerve-muscle preparation, &c., in the moist chamber as usual, draw first, if not ready at hand, the curve of a simple muscular contraction, for comparison.

Then connect the electrodes with the induction machine using the magnetic interruptor; insert between the secondary coil and the electrodes the marking key with double circuit; raising the marking key will now allow an interrupted current to fall into the nerve; on pressing the key down the current will be shut off.

All being arranged (the slow axis of Sécretan's instrument will give sufficient speed), allow an interrupted current of very moderate intensity (*i.e.*, the secondary coil hardly, if at all, overlapping the primary with a weak battery) to break into the nerve, and in a few seconds shut it off again.

A curve similar to that shown in fig. 282 will be obtained; where the plumb line *m* drawn through the first *a* marks the commencement both of the stimulation and contraction (the speed not being sufficient to show the latent period), and the line m^2 through the second *a* marks the end of the stimulation, and m^3 the end of the contraction.

It will be seen that the curve rises at first very rapidly,* afterwards more slowly, and speedily reaches a maximum, which it maintains during the whole time of the stimulation. Upon the cessation of the stimulus at m^2, the curve at once falls, at first very rapidly, but afterwards more slowly, and in its later phases very slowly.

If the stimulus is allowed to act upon the muscle for more than a few seconds, the curve begins to decline, even while the stimulus is still acting; but, even after very prolonged stimulation, the cessation of the stimulus is indicated by a sharp fall in the curve.

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* In the figure the curve does not rise rapidly enough.

Tetanus from an ordinary interrupted current is a continued contraction rapidly reaching a maximum, continuing (within limits) in that condition so long as the current is passing, and followed by a gradual relaxation upon the current being cut off.

Obs. II. Arrange for a single induction coil, but replace the key *b* by the oscillating interruptor (Chap. XIX, sec. XIII.). Use the first or the second axis of Sécretan, and the needle of the interruptor being clear of the mercury, open the key *a*, and set the cylinder revolving. When uniformity of speed has been reached, suddenly set the interruptor vibrating, and after some ten vibrations or so have taken place, close the key *a*.

The tracing on the cylinder will be a curve of the character shewn in fig. 283.

In general features it resembles the curve, fig. 282. There is the same rise, maximum, and fall; but instead of being, as in fig. 282, apparently a simple curve, it is evidently composed of a series of curves. Each of these component curves corresponds to a contraction caused by a breaking or a making of the primary current through the needle dipping into or coming out of the mercury. It will be seen that the second contraction began before the first was completed, and is, so to speak, placed on the top of it; in the same way, the third comes on the top of the second, and so on. The amount of rise contributed by each subordinate curve to the total rise is greatest in the first, and goes on diminishing until the maximum is reached.

By varying the length of the oscillating slip, a series of curves may be obtained, showing the various steps between a series of quite separate contractions, each being completed before the next begins, and one in which (as in the tetanus produced with the magnetic interruptor) the individual contractions follow each other so rapidly, that no trace of their separate existence is visible on the recording surface.

Tetanus really consists of a series of simple muscular contractions fused together.

II. The Effects of Exhaustion.

Obs. III. Throw a muscle, with the electrodes applied to the muscle itself, into tetanus, with a strong interrupted current. Record the movement on the cylinder. Continue the current for some minutes. The curve will gradually fall from the maximum down to very nearly the abscissa line; but even after very prolonged action, a sudden fall will mark the shutting off the current.

Obs. IV. Send through a muscle a single induction shock of a certain strength. Record the contraction. Then tetanize the muscle by means of the interrupted current for ten or twenty seconds.

Apply again the same induction shock as before. There will be either a much slighter contraction than before, or none at all. After waiting some minutes, repeat the shock again. The contraction will now be much nearer its former dimensions.

By contraction, especially by tetanus, irritability of a muscle is diminished; after a period of rest, the irritability returns even in a muscle removed from the blood current.

Obs. V. Repeat the observation on a muscle still connected with the blood current. The return of irritability will be much more rapid and complete.

With a Du Bois Reymond's induction apparatus, the transition from a single induction shock to an interrupted current may easily be effected thus: The apparatus being arranged for an interrupted current, the key *a* being open, press the spring of the magnetic interruptor up to the platinum point, and open the key *b*. The current breaks into the primary coil, and a single (making or closing) induction shock is the result. On letting go the spring, an interrupted current is at once obtained. This may be stopped at any moment by pressing down the spring, and then a single shock is again obtained by letting it rise once against the platinum point, and keeping it there.

III. Phenomena attending Muscular Contraction.

These can only be satisfactorily determined by studying tetanus. The changes in a single contraction are too slight and transitory to be distinctly appreciated.

Obs. VI. *During contraction there is no appreciable change of bulk.*

Take the whole leg, or, better still, both legs, of a frog, including the attachment of the thigh muscles to the ilium and coccyx, and remove the skin. Tie a thin platinum wire round each end of the leg. Place the thigh in a bottle filled with normal saline solution, insert a cork in the mouth, bring the platinum wires through the cork, and in the centre of the cork insert a narrow glass tube. Fill the tube up to a certain height with the saline solution, make sure that no air bubble remains below the cork or entangled in the leg, and that the cork is tight. Place a scale behind the glass tube in order that the level of the solution may be exactly determined, and bring the platinum wires into connection with an induction coil arranged for an interrupted current.

Tetanize the leg with a strong current; even at the height of the tetanus, no perceptible change of level in the fluid in the tube will take place.

Obs. VII. *During contraction the elasticity of the muscle is diminished, i.e., its extensibility is increased.*

Load a muscle with 50 grammes and record the amount of extension. Remove the load and tetanize the muscle. At the height of tetanus, load the muscle again with the 50 grammes and record the extension. This will be found to be much larger in the second instance than the first. If tracings of the extension be taken on a revolving cylinder, curves similar to those shown in fig. 284 will be obtained. When the muscle is at rest and unloaded, the recording point of the lever describes the straight line *o, x*. The sudden application and speedy removal of the load produces the curve *x, a*, the muscle in this instance failing to return to its original length. On being tetanized the muscle shortens from the level of *x′ a* to the level of *o*; and the application of the same load as before produces the long curve *o′ a′*.

Obs. VIII. *During contraction there is a diminution, a negative variation, of the natural muscle current.*

This is shown by the galvanometer (*see* Chap. XXV., sec. II.).

It may also be shown by using the variations in the muscular current, as a means of stimulating a nerve supplying another muscle.

Get ready two nerve-muscle preparations as irritable and as little injured as possible; one may be the whole of the under leg, with the femur cut off close to knee, and as long a sciatic nerve as possible (fig. 285 A); the other should include the muscles of the thigh as well, the skin being in both cases removed (fig. 285 B).

Place B on a glass plate, and let the extreme (central) end of the nerve rest on a pair of electrodes connected with an induction coil.

Lay the nerve of A over the muscles of the thigh of B, as in the figure.

Send a single induction shock through B; there will be a single contraction of the muscles of B, and almost at the same time a single contraction of the muscles of A.

Send an interrupted current through the electrodes of B. The muscles of B will be thrown into tetanus. So also will those of A.

The single contraction of the muscles of B causes a single variation in the natural currents of the muscles of B; this acts as a single stimulus to the nerve of A, and so causes a single contraction in the muscles of A.

When the muscles of B are thrown into tetanus, each constituent contraction of which that tetanus is made up causes a corresponding variation in the natural current, which therefore during the tetanus is undergoing a succession of variations. Each such variation acts as a stimulus to the nerve of A, and accordingly the muscles of A are thrown into a tetanus, the constituent contractions of which correspond exactly with those of the muscles of B.

In the galvanometer we have no such series of variations in the position of the needle; the negative variation during tetanus appears as a steady backward swing of the needle. This is because the inertia of the needle prevents its responding with sufficient rapidity to the variations in the current.

The proof that the negative variation of tetanus is thus really made up of a succession of variations is supplied, not by the galvanometer, but by the above experiment with the frog's muscle, or, as it is often called, the "*rheoscopic frog*."

The above observation will frequently fail unless the nerves are perfectly fresh and irritable.

Obs. IX. Satisfactory results having been obtained, ligature tightly in one case the nerve of B between the muscles and the electrodes, and in another the nerve of A between its muscles and the part of the nerve lying on the muscles of B.

In either case, the secondary contractions in A should be entirely absent. If they are present, they are due to an escape of the current; and the observation must be repeated on fresh muscles and nerves, greater precautions being taken to prevent the escape of the current.

Obs. X. *During contraction, muscle becomes acid.*

Prepare two muscles, either the gastrocnemius or rectus, or, perhaps better still, take the whole of the thigh muscles. Leave one, A, alone; tetanize the other, B, repeatedly. Make an incision through each and test their reaction.

A will be found to be neutral or alkaline; B will be found to be distinctly acid.

Obs. XI. *During contraction, the temperature of the muscle rises.*

Prepare a whole leg with sciatic nerve; choose a large, healthy, strong frog. From the thigh resect the femur in its middle for the greater part of its length, injuring the nerve and muscles as little as possible.

In place of the removed femur, place the bulb of a thermometer reading one-tenth of a degree centrigrade at least; wrap the muscles carefully round the bulb; surround the thigh with cotton wool, and wait till the level of the mercury is constant. The thermometer should be fixed very firmly and steady. Now send an interrupted current through the nerve. The muscles will be thrown into tetanus, and the mercury in the thermometer will rise.

(For determining more exactly the changes of temperature in a muscle during contraction, it is better to use thermopile needles with a galvanometer of little resistance (*see* Chap. XVIII., p. 335); or for still finer observations, in which it is desirable to avoid the effects of friction, the swinging apparatus of Heidenhain may be employed. (*See Heidenhain-Mechan. Leistung, Wärmeentwickelung, &c., bei der Muskelthätigkeit.*)

CHAPTER XXV.

ELECTRIC CURRENTS OF MUSCLES.

Obs. I. **The Natural Currents.**—Place the galvanometer A and scale B east and west (with lamp lighted) about three feet apart, level the galvanometer with the levelling screws C, carefully set the mirror free if needful by gently raising the milled head seen on the top of the galvanometer when the glass cover is removed,* and adjust the height of the lamp by pulling in and out its brass neck, or moving it from side to side until the light falls well on the mirror.

The most convenient galvanometer for the purpose is Sir William Thomson's. The one represented in the figure (fig. 286) is a differential one, but should be used as a single one in the following observations by connecting the two central binding screws *a a* with a piece of wire.

Having put on the glass cover, screw the adjusting magnet *d* with its upright *e* on to the top of the galvanometer. Let the magnet, with its north pole directed towards the magnetic north, be at first at the top of the upright; gradually bring it down, moving it from side to side, and carefully watching the spot of light as it travels to and fro on the scale. Before the magnet has descended very far, the student will have so far gained command over the mirror, as, by moving the magnet to a certain position right or left, to be able to bring the spot of light nearly to zero.

This done, shift the scale away from or towards the galvanometer, until the image of the slit *f* through which the lamp shines is well focussed on the scale. (If not provided on the scale, affix an upright wire in the middle of the slit; have the slit wide, and use the shadow of the wire seen in the broad spot of light, to determine the position of the mirror.)

Now bring the magnet very gradually still lower down, keeping the spot of light as near as possible to zero, and watching attentively the rapidity with which the spot oscillates on either side of that point. It will be found that as the magnet descends the oscillations become slower and slower. This indicates that the influence of the earth's magnetism is becoming more and more neutralized by the magnetism of the adjusting magnet. On continuing to lower the magnet, the point of neutralization is soon passed, and then the influence of the adjusting magnet on the needle becomes stronger than that of the earth. The needle, consequently, which previously had its north pole under the north pole of the magnet, would, if free to turn, swing half round in the attempt to bring its south pole under the north pole of the magnet; and indeed does swing round as far as the arrangements of the apparatus will allow, the spot of light rapidly travelling quite beyond the limits of the scale. When this has been found to occur, the magnet must be raised again up to or rather above the point of neutralization. The oscillations of the needle will now be at their minimum of rapidity, and the needle will be at its maximum of sensitiveness. Bring the spot of light exactly to zero. The magnet may be at first moved with the hand, but this will soon be found to be too coarse a method. Finer adjustment is gained by turning the milled head *f*.

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* If possible, the galvanometer should be carefully levelled and set once for all, and kept so in some place where it need not be disturbed.

The wires conveying the current through the galvanometer are to be attached to the outer binding screws *b b*.

To determine which direction of current is indicated by the movement of the spot of light, try the effect of a very feeble cell upon the galvanometer. But be careful not to use the whole of the current proceeding from the cell; cut off the greater part of it by means of the shunt. (Fig. 287.)

Bring the wires from the cell to the binding screws of the shunt. With the plug placed in the hole between the binding screws, there is no resistance offered by the shunt. The whole current consequently flows through the shunt, none going through the circuit of the galvanometer. The shunt may thus be used as a key, and it will not be necessary to have another key between the galvanometer and the electrodes. If a plug be inserted in the hole marked 1-9, and the plug between the binding screws be withdrawn, such resistance is offered by the shunt, that one-tenth of the total current of cell goes through the galvanometer. Similarly with the plug in the hole marked 1-99, instead of in the hole marked 1-9, 1-100th goes through the galvanometer; so also with 1-999.

By means of the shunt send 1-1000th of the current from a cell through the galvanometer, and mark the direction in which the light travels. Note which screw of the galvanometer is connected with the kathode, and which with the anode, and the relation of the direction of travel of the spot of light to that of the current is known. Most probably it will be found that the light travels in the same way as the current.

Obs. II. Prepare two non-polarizable electrodes with truncated ends, or with the plug projecting; connect them with the shunt, using it as a key.

The plug being in the shunt and the spot of light at zero, place the two electrodes so that the plugs touch each other, or place a morsel of thread or paper, moistened with normal saline solution, over the two plugs, and open the key. The needle should remain at zero. If any deviation occurs, it is an indication of a current in the electrodes themselves. If the deviation is slight and constant, its direction and amount in degrees must be noted, and all subsequent observations corrected by it. This may be done by shifting the scale a little, so as to bring the spot of light to zero, or by bringing the spot of light to zero by means of the adjusting magnet. If it be large, a fresh pair of electrodes must be prepared, which shall give no such deviation.

Obs. III. The muscle may now be prepared. Take one of the large muscles of the thigh, *e.g.*, the triceps (fig. 267 *a*); with a sharp clean knife or scissors cut the tendon of insertion clear away with a transverse cut; similarly make a transverse cut at the origin. Place the muscle thus prepared on a glass plate with the electrodes under a moist chamber. The muscle will have a natural longitudinal surface and two artificial transverse surfaces. Place one electrode on the longitudinal surface at a point as near as possible midway between the two ends, and the other as near as possible in the centre of one of the transverse sections. Connect the electrodes with the binding screws of the shunt, the plug of the shunt being in place between the screws. Remove the plug. A deviation of the needle will take place. Most probably the spot of light will swing right out of sight beyond the limit of the scale. If this is so, replace the plug; when the needle has returned to zero, shunt by means of the second plug; for instance, put the second plug in the hole marked 1–99 and thus allow only 1-100th of the muscle current to pass through the galvanometer, and then remove the first plug. The deflection will be far less. Note its direction and amount (number of degrees of scale).

A current will be found passing through the galvanometer from the mid-point of the longitudinal surface to the central point of the transverse section. Replace the plug, so as to shut off all the current from the galvanometer.

Obs. IV. Keeping the one electrode still on the transverse section, shift the other electrode from the mid-longitudinal point to some point nearer that transverse section; remove the plug. The deflection of the needle will indicate a current in the same direction as before, but of less strength. Replace the plug.

Obs. V. Place the electrodes in the following positions, always replacing the plug (serving as key) between the binding screws of the shunt after each observation, and always being careful that the amount of contact between the electrodes and muscle is as nearly as possible the same in all cases :—

One electrode on the mid-longitudinal point, the other at the other transverse section. The current will be, as before, from the longitudinal surface to the transverse section.

Obs. VI. One electrode on or near the mid-longitudinal surface, the other at a point nearer either transverse section. The current will be slight, and its direction will be from the point on or near the mid-longitudinal point to the one farther off.

Obs. VII. The two electrodes on the longitudinal surface on either side at unequal distances from the middle point or equator. The current will be slight, and from the point nearer the middle to the point farther off.

Obs. VIII. The two electrodes on the longitudinal surface at equal distances from the middle point on either side; there will be little or no current at all.

Obs. IX. By using very pointed electrodes, evidence of a current may be obtained on the transverse section from the electrode farther from the centre to that nearer to the centre.

Obs. X. The student may repeat these observations on a muscle to which an artificial longitudinal surface has been given by a clean section, and also on a muscle, the tendons of origin and insertion of which have been divided without injury to the muscular fibres, *i.e.*, on a muscle with natural transverse surfaces as well as a natural longitudinal surface.

In all cases the following result will come out more or less clearly :

In any muscle, or piece of muscle, with natural or artificial longitudinal and transverse surfaces, evidence may be obtained of a current passing through the electrodes from the middle of the longitudinal surface (*from the equator*) *to the centre of either transverse section, and from any point nearer the equator to any point nearer the centre of either transverse section; the current is stronger the farther apart these two points lie* (*see* fig. 288, where the direction of the currents obtainable from a piece of muscle of rectangular form is indicated by the arrows, and the intensity by the sweep of the curves. The points *a a,* equidistant from the equator, give no current).

Obs. XI. Immerse the muscle on which you have been experimenting in water at 40°, in order to kill it. As soon as it is cool, repeat the above observations. No currents at all, or very trifling ones, will be obtained, if the muscle be perfectly and completely "rigid."

The currents obtainable from a living muscle disappear when rigor mortis is complete.

In all cases examine the electrodes by themselves, after any series of observations, as well as before, in order to be sure that no changes have taken place in them during the observations, such as would give rise to a current.

II. Negative Variation.—*Obs.* XII. Get ready a nerve-muscle preparation, and make a transverse section through the lower end of the muscle. Lay the muscle on a glass plate; connect the equator and transverse section of the muscle by non-polarizable electrodes with the shunt and so with the galvanometer; lay the end of the nerve (as far away from the muscle as possible) on another pair of electrodes. Connect this second pair, or "exciting electrodes," as they may be called, with an induction coil arranged for an interrupted current. Let the induction coil be as far as possible away from the galvanometer, and before commencing the observation ascertain that the setting the induction machine in action does not affect the needle.

The spot of light being at zero, remove the plug of the shunt, and when the spot has come to rest (using a shunt if the current is too great for the scale), send a moderately strong interrupted current through the exciting electrodes. The muscle will become tetanized; at the same time the spot of light will travel back a certain distance toward zero, *i.e.*, the current obtainable from the muscle at rest is diminished, or suffers a negative variation during tetanus. Shut off the tetanizing current; the needle returns towards its former position. If the muscle be laid flat on the glass plate, considerable tetanus may be called forth without the electrodes at all shifting their position in relation to the muscle, especially if they be pressed somewhat firmly on to the muscle to start with.

Obs. XIII. Having determined the negative variation as above, tie a piece of wet silk or thread tightly round the nerve between the muscle and the exciting electrodes, being very careful to disturb nothing else. Now send the same interrupted current as before through the exciting electrodes. There will be no tetanus and no negative variation. The ligature, having destroyed the vital continuity of the nerve, has prevented the passage of nervous impulses along the nerve to the muscle.

Should any influence on the galvanometer be observable, it is an indication that an escape of current from the exciting electrodes to the galvanometer electrodes has taken place. The ligature of the nerve does not destroy the electrical continuity of the nerve, though it does its vital continuity.

The exciting electrodes must be removed further from the muscle, or a weaker current used, so as to prevent this escape of current, and the observation then repeated.

CHAPTER XXVI.

ELECTRIC CURRENTS OF NERVES.

I. Natural Currents.—*Obs.* I. Bring the galvanometer into as sensitive a condition as possible. The shunt will be unnecessary in this case except to be used as a key. Prepare as long a piece of nerve as possible, with the least possible injury. Hang the middle of the nerve over a bent non-polarizable electrode, and bring both ends to rest on the plug of another electrode, as represented in fig. 289. In this way, one electrode will be in contact with the equator, and the other with both transverse sections. The current from the equator to each transverse section being the same in direction, the result of this arrangement will be to double the effect on the needle.

The current in the nerve, far feebler than that in muscle, is as in the muscle from the equator (*or mid-longitudinal point*) *to the transverse section.*

Obs. II. By doubling a long piece of nerve and laying different points on the electrodes, it may be determined that the arrangements of the currents are the same in one case as in the other. Naturally, the various points in the minute transverse section cannot be examined.

II. Negative Variation in Nerve.—*Obs.* III. Prepare as long a piece of nerve as possible; lay the transverse section of the central end and a point in the longitudinal surface at some little distance from that end on the pair of galvanometer electrodes. Lay any two points at the other end (peripheral) of the nerve on a pair of exciting electrodes connected with an interrupted current.

Determine the amount of deflection given by the natural current. Send an interrupted current through the exciting pair. There should be a slight but distinct diminution, a slight negative variation, of the current. *When a nerve is excited, the natural current suffers a negative variation.*

Obs. IV. Repeat the observation, placing the peripheral end of the nerve on the galvanometer electrodes, and the central on the exciting electrodes.

There will be, as before, a diminution, a negative variation of the current.

The negative variation travels along the nerve in either direction.

Obs. V. Ascertain as before, by ligature, that the effects witnessed are not due to any escape of current.

CHAPTER XXVII.

ELECTROTONUS.

Obs. I. Prepare as long a piece of nerve as possible. Get ready *two pair* of non-polarizable electrodes. Place the thicker (central) end of the nerve, on one pair of electrodes, *a a′* fig. 290. This figure is intended to represent diagrammatically the effects of a polarizing current, *p p′*, acting on the centre of a piece of nerve, as seen by testing either end with a galvanometer. It will serve, however, to illustrate the simpler case of *Obs.* I., if the electrodes *b b′* be supposed to be removed and *p p′* brought nearer to that end of the nerve. Let one electrode be on the transverse section of the nerve, and the other on the longitudinal surface at some distance, so as to obtain a tolerably good current. Connect this pair of electrodes with the galvanometer, putting in a key or using the shunt.

Place the other end of the nerve on the other pair of electrodes, *p p′*; connect these electrodes with a cell, which may be called the polarizing cell, interposing a commutator (Chap. XIX., sec. VII.). Cover the nerve with a shade, or put it with the electrodes in the nerve chamber (Chap. XIX., sec. IV.), to protect it against evaporation.

Both keys being down, and the needle of the galvanometer being at zero, open the key of *a a′*, and note the deflection of the needle. The current will of course pass through the galvanometer in the direction of the arrow in the figure from *a* to *a′*, and the circuit may be supposed to be completed by the current passing *inside the nerve* in the direction of the arrow. Shut the key of *a a′*.

Obs. II. Now open the commutator of the polarizing cell in such a way that the current of the cell passes from *p* to *p′*, in the direction of the arrows in the figure, *i.e.*, flows in the same direction as the natural nerve current flows through the galvanometer. Open the key of *a a′*. Note again the deflection of the needle; it will be found to be greater than it was before.

Shut the key of *a a′* and shut off the polarizing current. Then reopen *a a′*. The needle will be found to return to the position it had in *Obs.* I.

Obs. III. Repeat the observation, but reverse the polarizing current; let it flow from *p′* to *p*, that is, in a direction contrary to the natural nerve-current. The needle of the galvanometer will now be found to have suffered a diminution of deflection instead of an increase.

"*When a constant current is allowed to break into a nerve, the natural nerve current, even at some distance from the electrodes, is affected during the whole time of the passage of the constant (polarizing) current; when the natural and polarizing currents have the same direction, the natural current is increased; when contrary directions, the natural current is diminished.*"

This condition of the nerve, maintained during the whole passage of the current, is known as *electrotonus*.

Obs. IV. Tie the nerve very tightly with a ligature between the polarizing and the galvanometer electrodes; or divide it, and place the ends carefully in exact opposition, and repeat the observations. It will be found that the natural current is in no way affected by the polarizing current.

The phenomena, therefore, are not due to any escape of the battery current; something more than mere physical continuity is required for their development.

Obs. V. Repeat the observations, placing the thinner (peripheral) end of the nerve on the galvanometer electrodes, and the thicker on the polarizing electrodes.

The results are the same; electrotonus is established equally well in either direction.

Obs. VI. The same result may be better shown in the following way: Take three pair of electrodes. Place the polarizing pair *p p′*, fig. 290, in the middle of the nerve, and connect the other two pair with the two cut ends, as shown in the figure. Bring the wires from *a a′* to a key, and those from *b b′* to another key; then the wires from both keys to the same binding screws of the galvanometer. By opening the key of *a a′* while that of *b b′* is shut, or *vice versâ,* the amount of natural current in *a a′* or *b b′* may be respectively determined. (Or use the double key, as directed in Chap. XIX., sec. IX.) Determine both before the key of *p p′* is opened. Then open the key of *p p′* and determine the amount of deflection both in *a a′* and *b b′*.

It will be found that when the current passes from *p* to *p′* in the direction of the arrow, as in the figure, the current at *b b′* is *diminished* (in the neighbourhood of the *kathode*), while that at *a a′* is *increased* (in the neighbourhood of the *anode*). If the direction of the polarizing current be reversed, if it be made to flow from *p′* to *p*, then *a a′* will be diminished and *b b′* increased.

Obs. VII. Repeat the observation, placing the galvanometer electrodes, not at the cut ends as before, but on any two points from which a natural current can be obtained. Similar results will be observed.

With most dispositions of the electrodes the natural current is increased in the neighbourhood of the positive and diminished in that of the negative pole. The region of the negative pole is said to be thrown into *katelectrotonus,* that of the positive into *anelectrotonus.*

Obs. VIII. Having determined the amount of diminution of *b b′* and the amount of increase of *a a′* when the polarizing electrode is exactly in the middle line between the other two pair, shift the polarizing electrodes nearer to *b b′*. Be very careful that the electrodes in their new position are *exactly* the same distance from each other as before, and that the nerve touches the plugs of the electrodes exactly as before, so that the only difference established is that a different part of the nerve is placed between the electrodes. Be careful also not to disturb the position of the nerves on the two pair of galvanometer electrodes. If all has been done properly before the polarizing current is allowed to break into the nerve, the amount of deflection at *a a′* and *b b′* should be the same as when the polarizing electrodes were in the middle. Now open the key of the polarizing current and determine the deflection at *a a′* and *b b′*. The diminution of deflection at *b b′* should be greater, and the increase at *a a′* less, than when the polarizing electrodes were in the middle. Reverse the direction of the polarizing current. The increase at *b b′* will be greater, the decrease at *a a′* less, than when the electrodes were in the middle.

Obs. IX. Shift the electrodes (carefully as before) towards *a a′* instead of towards *b b′*, and repeat as in *Obs.* VIII. It will be found, as before, that the nearer the galvanometer electrodes are to the polarizing electrodes, the greater the effect either in way of decrease or increase, as the case may be, of the natural current.

The amount of electrotonic increase or decrease is greater, the nearer the points tested lie to the polarizing electrodes.

Obs. X. Having determined the amount of electrotonus established by the passage of a current from a single cell, use two cells (keeping everything just the same), and compare the results; then three cells; then four.

The amount of electrotonic increase or decrease of the natural current increases with an increasing intensity of the polarizing current.

Obs. XI. Determine the electrotonic increase and decrease with a given current on a perfectly fresh nerve from a strong frog. Allow the nerve to remain for some time exposed in the moist chamber, and repeat the observation. The electrotonic effects will be found to be less.

The amount of electrotonic variation is dependent on the vital conditions of the nerve.

CHAPTER XXVIII.

STIMULATION OF NERVES.

Other things being constant, we may now take variations in the contraction of the muscle of a nerve-muscle preparation as a measure of variations in the condition of the nerve. A muscular contraction is a token of a nervous impulse passing along the nerve, the extent and character of the one being a measure of the extent and character of the other: a tetanus in the muscle indicates a series of impulses in the nerves, following each other with not less than a certain velocity.

I. The Effects of the Constant Current.—*Obs.* I. Arrange a nerve-muscle preparation in the moist chamber, with the nerve on non-polarizable electrodes, the muscle loaded with 10 or 15 grammes, lever attached, recording surface prepared, &c.

Have a battery of two or more cells, and between the battery and the electrodes introduce the rheochord (Chap. XIX., sec. VIII.). Let all the plugs be in, and the travelling mercury cups close up.

The resistance now offered by the rheochord, compared with that offered by the electrodes, is practically nil; consequently none of the current from the battery will pass through the latter; there will therefore be no contraction in the muscle.

Remove one of the plugs, viz., that one the removal of which throws the least resistance into the rheochord. A certain fraction of the current will now pass through the electrodes on account of the resistance thrown into the current through the rheochord by the removal of the plug. If a contraction be the result, let it be recorded; if none, let that fact be recorded too, noting on the recording surface the plug removed. Remove the plugs one by one, recording the result each time. Replace the plugs one by one, also noting the results.

It will be found that a contraction of the muscle takes place, a nervous impulse is originated, only at the moment when the plug is withdrawn or replaced. It may be present at both withdrawal or re-insertion, or at either or at neither; but no contraction occurs in the interval during which the plug or plugs remain away from the board or in their place, provided that the current in the battery be constant and the condition of the nerve-muscle normal.

A nervous impulse is generated in a nerve only when there is a sudden change in the intensity of a constant current passing through it (including the changes from and to zero, i.e., *the total breaking and making of the current). So long as the current remains uniform in intensity, there is no contraction of the muscle, no nervous impulse generated in the nerve.*

The contractions so obtained are simple contractions, indicative of the advent of a single nervous impulse. Very often, especially in working with winter frogs in early spring, the contractions thus obtained by variations in the intensity of a constant current are not simple, but *tetanic.* This is an abnormal result, which has not yet been investigated.

The contractions obtained above are not only variable, inasmuch as they come either at a diminution (breaking) or increase (making) of the current, or at both, but also differ in extent, *i.e.,* the nervous impulses differ in intensity.

These variations depend on the strength of the current (amount of variation of the current), the direction of the current, and the condition of the nerve.

II. Law of Contraction.—*Obs.* II. Arrange in the moist chamber a nerve-muscle preparation as fresh and lively as possible. Place the nerve on a pair of non-polarizable electrodes, about a centimètre apart. Insert between the electrodes and a battery of two or more cells, first the commutator, and then the rheochord. Let the positive and negative wires have different colours, the same throughout the whole apparatus in each case, and arrange so that when the handle of the commutator is *raised,* the current is *ascending* in the nerve; when *depressed, descending.*

The handle of the commutator being horizontal, and the plugs of the rheochord all in, withdraw the mercury cups a few degrees of the scale, and depress the handle of the commutator. If there be any contraction, record it. This is equivalent to the making in the nerve of an extremely feeble descending current. Then bring the handle of the commutator horizontal, and so break this feeble current, recording any result.

After waiting a few minutes, repeat the observation, using an ascending current instead of a descending. Thus will be obtained the effects of breaking and making an extremely feeble constant current both ascending and descending.

Then shift the mercury cups several degrees, and repeat the whole observation. This will give the effects of making and breaking a still feeble but yet rather stronger descending and ascending current.

Proceed in this way, shifting the mercury cups by stages, until they are brought to the other end of the board; then remove the plugs one by one, the removal of each plug marking a corresponding augmentation of the strength of the current sent through the electrodes on the nerves.

Wait some minutes between each observation to allow the nerve to recover itself. Tabulate the results. They should be such that, throwing the various intensities of current into four categories, they illustrate the following law:—

	Descending.		Ascending.	
	Make.	Break.	Make.	Break.
Weakest	Yes	No	No	No
Weak	Yes	No	Yes	No
Moderate	Yes	Yes	Yes	Yes
Strong	Yes	No	No	Yes

where "Yes" means a contraction; "No," none.

The *making* of the *descending* current is the first to make itself manifest by its effects, and maintains its pre-eminence throughout the series as the most certain and strongest stimulus.

Next, the *making* of the *ascending* current also becomes efficient; then the *breaking* of the *descending*; lastly, the *breaking* of the *ascending*; so that with a certain intensity of current which we here call "moderate," a contraction is called forth both by making and breaking both ascending and descending currents.

With a further increase of intensity, the contraction which follows upon the *making* of the *ascending* current gets less, and finally disappears altogether. The contraction due to *breaking* the *descending* current suffers subsequently the same fate, so that with a "strong" current we have only a single contraction with each current; but it is a contraction on *making* in the case of the *descending*, on *breaking* in case of the *ascending*.

We have seen that when a constant current is sent into a nerve, katelectrotonus is established at the negative pole, anelectrotonus at the positive. Both conditions remain during the whole time of the passage, and both disappear (with more or less rebound) when the current is broken.

It is evident from the above observations that the rise of a nervous impulse is connected with the transition of a nerve from its ordinary condition into that of either katelectrotonus or anelectrotonus, or both, or with its return from katelectrotonus or anelectrotonus into its normal condition, and not with its being or remaining in either katelectrotonus or anelectrotonus.

Further, it is evident from the different results of breaking and making, that the entrance into katelectrotonus and anelectrotonus has not the same relation to the origin of a nervous impulse as has the exit from those states.

Lastly, from the different behaviour of the ascending and descending currents, it appears that the effect of the entrance into katelectrotonus is not the same as that of the entrance into anelectrotonus, and that the effects of the exits from those states likewise differ.

III. Electrotonus as affecting Irritability.—Arrange a nerve-muscle preparation in the moist chamber, with lever, &c. Prepare two pair of non-polarizable electrodes. Place the end of the nerve on one pair, about 1 or 2 cm. apart; connect this, *the polarizing* pair, with a battery of one or two cells, the commutator intervening. Place the second pair between the first pair and the muscle, and connect this, the exciting pair, with an induction coil.

When the polarizing current is made a descending one, the portion of the nerve on which the exciting electrodes rest, will be in the region of *katelectrotonus;* when ascending, in *anelectrotonus.*

Obs. III. The polarizing current being shut off (the handle of the commutator horizontal), pass a single induction (opening) shock through the "exciting" pair, and record the contraction. Shift the secondary coil, if necessary, until a contraction of moderate excursion is obtained, and note the distance of the secondary coil from the primary.

Now let the polarizing current *ascend* in the nerve (through the polarizing pair of electrodes); the exciting pair will accordingly now be in the region of *anelectrotonus.*

Neglect the contraction which may be caused by the making (and subsequent breaking) of the constant polarizing current; and while the current is thus passing in an ascending direction, send a single induction shock of the same strength as before through the exciting pair, and record the contraction.

Shut off the polarizing current, and after a few minutes' rest, send a third time the same induction shock through the exciting pair.

Of the three contractions thus called forth by the same stimulus (the induction shock) under different circumstances, it will be found that the second is much smaller than the first, but the third nearly of the same size (it may be larger) as the first.

During the passage of a constant current, the irritability of a nerve is lessened in the anelectrotonic region, the same stimulus giving rise to a weaker nervous impulse, and so to a smaller contraction.

Obs. IV. Shift the secondary coil until it reaches such a position that the induction shock given becomes the minimum stimulus required to produce a muscular contraction, that is, any further removal of the secondary from the primary coil will lead to the absence of all contractions. This minimum stimulus then giving, in the absence of the polarizing current, a slight but obvious contraction, send an ascending polarizing current through the nerve; the contraction will be wholly absent. Remove the polarizing current, and excite again; the contraction will again make its appearance.

Obs. V. Remove the secondary coil a little further away from the primary, so that an induction shock gives no contraction where the polarizing current is cut off from the nerve. Pass a *descending* current through the polarizing pair, *i.e.*, throw the portion of nerve on which the exciting pair rest into *katelectrotonus.* Again pass the same induction shock as before: a contraction will follow.

Shut off the polarizing current, and after waiting a few minutes, send the induction shock through the exciting pair a third time. No contraction, or at best, a very slight one, will be obtained.

During the passage of a constant current, the irritability is increased in the region of katelectrotonus.

Obs. VI. The other arrangements being the same, put the magnetic interruptor into connection with the primary coil. Record the movements of the lever on the revolving cylinder.

With a not very strong interrupted current, throw the muscle into tetanus, and as soon as tetanus is established, send an ascending current through the polarizing electrodes for a few seconds only, and afterwards close the key of the interrupted current.

The curve of the tetanus on the recording cylinder will exhibit a marked fall (down even to zero if the polarizing current be strong enough) at the moment when the polarizing current breaks into the nerve, and a corresponding rise when the polarizing current is shut off.

This is simply another way of showing the diminution of irritability in anelectrotonus.

Obs. VII. Repeat the observation, using a very weak tetanizing current, and let the polarizing current be descending. The making of the polarizing current will be marked by a rise, and the breaking by a corresponding fall in the tetanus curve, indicating, as before, an increase of irritability in katelectrotonus.

Obs. VIII. Ligature the nerve between the two pair of electrodes, and repeat all the observations. The polarizing current will have no effect at all upon the results of the exciting current. Otherwise, part of the effects described above will have been due not to vital changes in the nerve, but to escape of current or simple electrical changes.

Obs. IX. Having arranged a nerve-muscle preparation with the polarizing, but without the exciting pair of electrodes, let the nerve between the electrodes and the muscle hang down in a loop.

Let the extreme end of the loop dip into a drop of concentrated solution of common salt. As soon as the irregular tetanic contractions resulting from the action of saline fluid on the nerve make their appearance, pass an *ascending* current through the electrodes. The tetanic spasms will be much lessened or cease altogether.

Pass a descending current through the electrodes; the spasms will be increased.

The *general irritability*, therefore, of the nerve is affected in electrotonus not simply its susceptibility to electrical modifications.

Obs. X. By introducing a rheochord between the battery and the polarizing electrodes and by varying the number of cells used, the student will ascertain that the *amount* of increase of irritability in katelectrotonus and decrease in anelectrotonus depends on the strength of the polarizing current, being greater with the stronger.

Obs. XI. By placing the polarizing electrodes at a variable distance from each other, it will be found that, with the same strength of current, the effect is greater the longer the piece of nerve between the polarizing electrodes.

Obs. XII. By shifting the exciting electrodes nearer to and farther from the polarizing electrodes, it will be found that the effects of both anelectrotonus and katelectrotonus are greatest in the immediate neighbourhood of the polarizing pair, and diminish the farther the exciting pair is from the polarizing.

In all the above observations, the stimulus, whether electric or chemical or other, is brought to bear on the nerve between the polarizing pair and the muscle.

Obs. XIII. They may be repeated with the polarizing pair placed between the exciting pair (or chemical stimulus) and the muscle. An ascending current will now throw the region of the exciting pair into katelectrotonus, a descending into anelectrotonus.

The general results will be the same, but they will not come out with the same distinctness, for the following reason. When the exciting pair is placed nearer to the muscle than the polarizing pair, the nerve between the exciting pair and the muscle is simply in a state of katelectrotonus, the intensity of which diminishes towards the muscle onwards. There is nothing between the exciting electrodes and the muscle to modify the increase of impulse due to katelectrotonus. When the exciting pair is on the other side of the polarizing pair, and the region of the exciting pair thrown into katelectrotonus, for instance, the increased impulse due to katelectrotonus after passing through the region of katelectrotonus, has to make its way through a region of anelectrotonus before it can reach the muscle,—it has to struggle in this region against antagonistic influences, and whether it reaches the muscle as an impulse greater than or less than, or simply equal to, that which occurs in a nerve not electrotonized, will depend on the relative amounts of the katelectrotonic increase of irritability, and the anelectrotonic decrease of conductivity.

This will be found to depend largely on the intensity of the polarizing current.

If the current be weak, the katelectrotonic increase over the normal impulse (of the non-electrotonized nerve), though lessened by having to pass through an anelectrotonic region will be evident as a larger contraction in the muscle.

If the polarizing current be strong, the contraction caused by the impulse originated in the katelectrotonic region will not only not be greater than the normal but will even be less, or may be absent altogether with a very strong (three or four Grove cells) polarizing current, owing to the impulse being completely blocked in the anelectrotonic region.

Mutatis mutandis, the same results are witnessed when the effect of an anelectrotonic decrease has to pass through a katelectrotonic region on its way to the muscle.

Obs. XIV. By placing the polarizing electrodes sufficiently far apart from each other, the exciting pair may be inserted into the *intrapolar* region, and the following results obtained.

In the intrapolar region, as in the extrapolar, there is an increase of irritability in the neighbourhood of the negative, and a decrease in the neighbourhood of the positive pole.

The increase and decrease respectively are greatest close to the poles, and diminish towards a neutral point situate between the poles.

With a weak current, this neutral point lies rather nearer to the negative pole than the positive. By increasing the strength of the current it is driven nearer and nearer to the positive pole.

IV. Other Variations in Irritability.—*The farther from the muscle the part of the nerve excited, the greater the contraction.*

Obs. XV. Arrange a nerve-muscle preparation with two pair of electrodes, one close to the muscle, the other near to the cut end of the nerve.

Connect both electrodes with a double key (Chap. XIX., sec. IX.), and the double key with an induction coil. Arrange for single opening induction shocks.

By means of the double key, put the lower electrodes next the muscle in connection with the secondary coil, and find what strength of current (what position of the secondary coil) just falls short of causing a contraction.

Then connect the upper electrodes with the secondary coil, in place of the lower ones. Send through these a shock of the same strength as that which sent through the lower electrodes produced no contraction. A distinct contraction will follow.

Once more send the same shock through the lower electrodes. There will be, as before, no contraction, or a very slight one.

The same stimulus produces, therefore, more effect when applied to a point farther from the muscle.

Obs. XVI. This is partly due to the section of the nerve trunk above the higher electrodes.

Having thoroughly destroyed the spinal cord of a frog, and laid bare the sciatic nerve without dividing it, place a pair of electrodes under the main sciatic trunk, send a feeble single induction shock through them, and record the amount of contraction in the gastrocnemius, or determine the position of the secondary coil, which gives a shock just falling short of the strength required to cause a contraction.

Divide the sciatic nerve a little distance above the electrodes, and determine, at intervals of 15 minutes, the contractions which result from the application of the same stimulus as before; or determine the position of the secondary coil for a minimum stimulus.

It will be found that the effect of the section is *first to increase*, and *afterwards to diminish*, the irritability of the portions of the nerve lying immediately below the section.

In the above observations, the student must make sure that the electrodes are exactly similar, so that the differences which come out are not due to any differences of resistance in the two pair of electrodes or to the electrodes of one pair being further apart from each other than those of the other, &c.

For this purpose it will be as well, after a series of observations, to exchange the electrodes, putting the one pair in the former position of the other, and repeat the series.

Obs. XVII. On the sciatic nerve of a frog in which the brain and spinal cord have been destroyed, and the heart removed so as to stop the circulation, place three pair of electrodes, one near the gastrocnemius, another close to the central end of the nerve, and a third between the other two. Divide the nerve above the upper pair.

Arrange the preparation carefully in the moist chamber. Send a single weak induction shock through each pair of electrodes, and record the contraction; or determine the minimum stimulus for each pair of electrodes.

Repeat the observation at intervals during the day.

It will be found that after the temporary increase due to section, the irritability gradually diminishes from the central cut end towards the periphery, the extreme muscular branches being the last to die.

Be careful that no part of the nerve is more exposed than others.

Obs. XVIII. Repeat the observation in a frog whose brain and spinal cord have been destroyed, but the blood current not interfered with.

The irritability will disappear much more slowly, but in the same centrifugal manner.

CHAPTER XXIX.

PHENOMENA ACCOMPANYING A NERVOUS IMPULSE.

The only phenomenon definitely and certainly known to accompany the passage of a nervous impulse is the negative variation of the nerve current (*see* Chap. XXVI., sec. II.).

In the case of muscle, the negative variation shown in tetanus by the galvanometer was proved by the rheoscopic frog to consist of a series of successive negative variations (*see* Chap. XXIV., sec. III.).

At first sight a similar proof seems to be afforded by the behaviour of nerves.

Obs. I. Prepare a nerve-muscle, and also a separate piece of nerve as long as possible. Place the nerve-muscle B (fig. 291) on a glass plate; place the nerve A over the nerve of B, in either of the positions shown in fig. 291 I. II.; connect the end of A with an induction coil.

A single shock sent through A will produce a contraction in B; an interrupted current will throw B into tetanus.

Obs. II. Ligature A between the electrodes and the end touching B. No contractions will appear in B on sending shocks through the electrodes. This proves that the results of *Obs.* I. were not due to any simple electrical conduction through A or to any escape of the current to B by other means.

The same thing is shown in the so-called "paradoxical contraction."

Obs. III. In the leg of a frog, the sciatic nerve divides at the lower end of the thigh into the *peroneal* and *tibial* branches. Dissect out one, say the peroneal, and divide it at its periphery. Divide the sciatic trunk high up, and place the peroneal branch on the electrodes of an induction coil. This will virtually convert the leg into a preparation similar to fig. 291, III.; the peroneal and tibial branches running, so to speak, side by side in the sciatic trunk.

Irritating the peroneal nerve A, with an interrupted current will produce contractions in the muscles to which the tibial B is distributed.

All these "secondary contractions" cease when the nerve A is ligatured between the electrodes and the nerve B.

With each making (competent to give rise to a nervous impulse) of the exciting current through A, two events take place which must be kept distinct in the mind of the student.

First, there is the electrotonic increase (in the anelectrotonic region) or decrease (in the katelectrotonic region) of the natural nerve current. This increase or decrease remains during the whole time of the passage of the exciting current, and disappears with the breaking.

Secondly, there is the *negative variation* of the natural current which travels with the nervous impulse indifferently in either direction, and which, in any given point of the nerve, is over and gone in an exceedingly short time after the act of making the exciting current.

During the time of the passage of the (uniformly constant) current, there is no negative variation, as there is no nervous impulse

On breaking the exciting current, a fresh negative variation sweeps along the nerve, if the current is of such a character that the breaking of it gives rise to a nervous impulse.

With a single induction shock there is also the double event of a negative variation, and, as well, of a momentary electrotonus; with an interrupted current there is a succession of such double events.

In both these cases the secondary contraction, as in *Obs.* I., II., III., may be due to either half of the double event: to the negative variation, or to the electrotonic change; or to both. To which of them it is really due cannot be decided by the use of such currents only.

If, however, the electrotonic increase is itself competent to cause a secondary contraction, the contraction ought to be obtainable at any period during the passage of an exciting constant current, at a time when the negative variation is absent.

Obs. IV. Connect A (placed on a glass plate) with a constant current of two cells, the positive pole towards the long free end; suspend the nerve of B in such a manner over A that, when desired, it can be let fall so as to lie upon A in the position I. or II. (fig. 291).

The exciting current being made, a negative variation sweeps over A and is gone. There remains, however, the anelectrotonic increase of the natural current of A along the whole region from the positive pole to the free end. Now let fall B as directed. A contraction in the muscle of B will follow.

This can only be due to the electrotonically increased natural nerve current of A acting as a stimulus to the nerve of B when the circuit is closed by a portion of B, and so causing a nervous impulse just as the closing of any other galvanic current would.

And inasmuch as the electric intensity of the electrotonic increase (or decrease) is much greater than that of the negative variation, the secondary contractions in the *Obs.* I., II., III. are chiefly due to this cause.

CHAPTER XXX.

VARIOUS FORMS OF STIMULATION OF MUSCLE AND NERVE.

I. Mechanical Stimulation.—A blow, sufficiently strong and sudden, applied to either muscle or nerve, will produce a contraction; and a series of such blows repeated sufficiently rapidly will produce a tetanus.

This may be roughly shown by striking simply by hand, with some thin but blunt instrument, either muscle or nerve.

For more exact purposes, the tetanomotor of Heidenhain may be used, and can be applied equally to muscle or nerve. For a description, *see* Rosenthal, Electricitätslehre, p. 116.

A simpler method is that of Marey's, with a tuning-fork.

Obs. I. Get ready a nerve-muscle preparation. Place the nerve on a small piece of india-rubber sheeting stretched quite tight over a ring of wood or metal. The object of the elastic india-rubber is to soften the violence of the blows given. Arrange a tuning-fork on a stand, in such a position that the vibrations of the tuning-fork shall take place at right angles to the nerve. Set the fork going, and bring it in slight contact with the nerve. The muscle will at once be thrown into tetanus, which may be recorded on the cylinder.

Obs. II. A muscle (gastrocnemius, or better, one of the recti) of a frog poisoned with urari, may be placed on the caoutchouc in place of the nerve.

Tetanus will be then obtained by direct mechanical irritation of the muscle itself, without intervention of the nerves.

II. Idio-Muscular Contractions.—*Obs.* III. Place on some flat surface a nerve-muscle preparation which has been much exhausted by treatment or by long removal from the body.

Strike the muscle sharply with some thin but blunt instrument (handle of scalpel), across the middle of the belly, at right angles to its long axis.

A contraction will probably follow; a contraction which, as usual, travels along the whole length of the fibres.

When the contraction, however, has passed away, the line where the blow fell will be marked by a wheal, *i.e.*, by a local shortening and thickening, which lasts for several seconds, but finally disappears. This wheal, this local thickening and shortening, is the idio-muscular contraction.

Obs. IV. Wait till neither muscle nor nerve give any (ordinary) contraction with an electric stimulus. Strike as before; the idio-muscular contraction will still make its appearance. The relaxation becomes slower the nearer the advent of *rigor mortis,* with the onset of which the idio-muscular contraction disappears.

III. Chemical Stimulation of Muscle.—*Obs.* V. Carefully dissect out the sartorius muscle in the front of the thigh (fig. 278 *s*), injuring it as little as possible, and taking away with it a piece of the pelvis from which it has its origin. Clamp the piece of pelvis, avoiding any entanglement of the fibres of the sartorius itself, and attach the clamp to a stand so that the muscle hangs vertical. If it be desired to record the contractions, thrust a fine needle through the middle of the muscle, and either bring the needle to bear directly on the recording surface, steadying it with a shotted thread as in the kymographion (Chap. XVI., § 33), or make the needle part of a delicate lever. With a sharp pair of scissors, cut off the tendon of insertion so as to lay bare a transverse section of muscular fibre.

Place a drop of any or each of the below-mentioned fluids on a rather greasy glass plate (so as to have a good convex surface of fluid), and very gradually raise the plate until the fluid comes in contact with the muscular surface. Immediately, or very shortly after contact, spasmodic contractions of the muscle will begin.

The following substances applied directly to muscular fibres produce contractions:—

Mineral acids, even when extremely diluted; solutions of metallic salts; strong solutions of neutral salts of the alkalis; lactic acid; glycerin, even diluted to a considerable extent.

Obs. VI. The vapour of ammonia, even in mere traces, acts as a powerful stimulus. Place a few drops of ammonia in a small, flat, wide-mouthed bottle; cover the top with a greased glass plate. Protect the muscle from all extraneous vapour of ammonia, and bring the closed bottle immediately under it. The muscle exhibiting no contractions (there being no escape of ammonia), slip away the glass cover from the top of the bottle; contractions will at once follow.

In the above observations, a fresh surface of muscle must be cut after each trial, as the body used as stimulus destroys the layer of muscle with which it is immediately in contact.

Apply the substance under trial as soon as possible after making the section, as the surface exposed soon dies.

IV. Chemical Stimulation of Nerve.—*Obs.* VII. Prepare a nerve-muscle with as long a piece of nerve as possible. Fasten the muscle in the clamp, and support the nerve so that the end hangs freely down in a vertical position. Bring a drop of one of the below-mentioned fluids, on a glass plate, in contact with the end of the nerve, allow some millimeters at least of the nerves to be fully immersed in the fluid; and either take a fresh nerve-muscle for each experiment or cut away each time all that portion of the nerve which had been previously exposed to the action of the fluid.

The movements of the muscle may be recorded as usual. Do not load the muscle with anything more than the lever itself.

The following substances applied to a nerve produce contractions in its muscles:—

Mineral acids, in considerable concentration *only;* neutral salts of the alkalis and metallic salts, in considerable concentration *only;* lactic acid, *only* when concentrated; glycerin, *only* when concentrated.

Ammonia hardly acts at all as a stimulus to nerve; in making trial with this, care must be taken to protect the muscle from all ammonia vapour.

V. Thermal Stimulation of Muscle.—*Obs.* VIII. Having arranged a sartorious muscle, as in *Obs.* V., bring to the lower cut surface a thin slip of heated metal. On contact taking place, a contraction will result. In this case the heat is applied to a part only of the muscle.

Obs. IX. Attach a gastrocnemius to a lever (either with the origin of the muscle downwards and the tendon upwards, or in the ordinary position with the tendon playing round a pulley) in such a way that the whole muscle may readily be immersed in fluid. Fig. 292 represents a convenient arrangement for this and other purposes. The muscle *a* is fastened to the clamp *c*, which is part of the bent holder *d*. This holder moves on the same upright as the lever *e*. The tendon of the muscle is attached by the thread *b* to the lever, so that its contractions pull the lever down. The lever is counterbalanced by weights carried over a pulley. The muscle can thus be readily immersed in or withdrawn from any fluid. Counterbalance the lever with 10 or 15 grammes.

Immerse the whole of the muscle in a small vessel filled with normal saline solution, and around the small vessel place a large one, through which send a stream of hot water.

By means of a thermometer, ascertain the temperature of the saline solution close to the muscle. When the temperature rises to 38°—40° C, the muscle is thrown into tetanus. In this case the temperature of the whole muscle has been raised at as nearly as possible the same time.

Immediately that tetanus has set in, withdraw the muscle from the saline solution. The tetanus will speedily pass away, and the muscle will remain alive and irritable.

Repeat the observation, but allow the muscle to continue at the temperature of 40° for about two minutes. On removing the solution, the muscle will still remain in a state of tetanic contraction, as indicated by the position of the lever, and from that contraction no relaxation will take place. No stimulus, however strong, will be able to call forth any further contraction. The reaction of the muscle will be found to be acid, and its extensibility diminished. In fact, the muscle will be found to have passed from a state of tetanus into a state of *rigor mortis*.

VI. Thermal Stimulation of Nerves.—*Obs.* X. Arrange the nerve-muscle preparation with the nerve dependent as in *Obs.* VII.

Bring a hot surface to bear on the end of the nerve, or dip the end of the nerve into a hot normal saline solution, or place the end of the nerve in a small quantity of the normal saline solution, the temperature of which gradually raise.

In all cases contractions in the muscle will follow.

CHAPTER XXXI.

URARI POISONING AND INDEPENDENT MUSCULAR IRRITABILITY.

Obs. I. Introduce beneath the skin of the back of a strong frog a drop or two of a solution of urari. (The exact strength of the solution and the dose required will depend on the source from which the urari has been obtained.) In a short time the frog will be found perfectly motionless, with its respiration arrested, but its heart still beating.

Lay bare the sciatic nerve in the thigh, slip under it a pair of electrodes connected with an induction coil, and stimulate the nerve with an interrupted current, taking care that there is no escape of the current into the surrounding muscles. This may be effected by slipping under the electrodes a small piece of india-rubber sheeting.

If the animal has been thoroughly poisoned, no contractions whatever in the muscles of the leg will follow upon the application of a stimulus, however strong, to the nerve. If contractions do make their appearance, the poisoning is not complete; and the student must wait or inject a further quantity of the poison.

The nerve having been proved to be insensible to stimuli, lay bare any of the muscles of the leg and apply the electrodes directly to them. Contractions will be manifest upon the application of a very slight stimulus.

The effect of urari is to destroy (or suspend) the irritability of nerves but not that of muscles.

Obs. II. In a strong frog make an incision through the skin between the ilium and coccyx along the line *k*, *m* fig. 266. Cut cautiously through the ileo-coccygeal muscle (fig. 267 *d*) until the peritoneal cavity is reached. The three nerves (fig. 295 7′ 8′ 9′), which go to form the sciatic nerve, will come into view when the sides of the wound are held apart. Very cautiously, by means of a small aneurism needle, pass a thread under these nerves, putting it under from the outside and bringing it out again on the median side. Be very careful not to wound the blood vessels.

Repeat the same process on the other side, passing the same thread under the nerves of that side too, but putting it in at the median side and bringing it out at the outside. The thread will now be in the position of the line *o p q* in fig. 266, with the nerves of one side lying over it between *o* and *p*, and those of the other side over it between *p* and *q*. Tie the thread very tightly round the abdomen, so as to check entirely the flow of blood to the lower limbs. All this may be done under a slight dose of chloroform. The nerves thus form the only means of communication between the hind limbs and the trunk, the vascular communication being entirely stopped. Now inject a small quantity of urari into the back, and wait until the poison has had time to produce its effects in that part of the body to which alone it has access, viz., the part above the ligature.

The following facts may then be determined :—

Though there are no voluntary movements in the body, head, or fore limbs, some slight (voluntary ?) movements may sometimes be witnessed in the hind limbs.

Pinching, or otherwise stimulating, either hind foot may produce movements in either one or both hind limbs, but in no other part of the body.

Pinching, or otherwise stimulating, the skin of the head, fore limbs or trunk above the ligature may produce movements in the hind limbs, but in no other part of the body.

These facts are intelligible only on the hypothesis that the urari has destroyed (or suspended) the irritability of the motor nerves in that part of the body to which, by means of the blood current, it has had access, but has not destroyed the irritability of the sensory nerves or of the central nervous system. Pinching the skin of the fore limb gave rise to an afferent nervous impulse which, either by volition or by reflex action, gave rise in turn to efferent impulses which were unable to manifest themselves through the poisoned motor nerves of the fore limbs and trunk, but found vent through the unpoisoned motor nerves of the hind limbs.

In order to bring these results out well, the dose of poison must not be more than sufficient to poison the motor nerves. Subsequent or stronger action of the poison affects the central nervous system as well.

Obs. III. In a fresh, strong frog, lay bare the sciatic nerve on one side—say the right—in its lower course, place a ligature under it near where it divides into its two branches, and tie the ligature tightly round the leg above the knee. The circulation of the lower right leg will thus be completely arrested; but inasmuch as the nerve is not included in the ligature, there will be complete nervous connection between the right lower leg and the rest of the body. Poison with urari. As soon as the animal has come under the influence of the poison, determine the following facts :—

Complete absence of spontaneous movements, except perhaps some feeble stirring of the right lower leg.

Stimulation of the right lower foot may produce movements in the right lower leg, but will not produce movements in any other part of the body.

Stimulation of any part of body may produce movements in the right lower leg, but in no other part of the body.

If the two sciatic nerves be laid bare along their whole course, it will be found that stimulation, however strong, applied to the left sciatic nerve, produces no contractions whatever in the muscles to which its branches go; while stimulation, even slight, of the right sciatic nerve, whether applied above or below the level of the ligature, and even close up to the spinal cord, produces contractions in the muscles of the right lower leg, but in none other.

Now the whole of the trunk of the right sciatic nerve, being supplied with poisoned blood, has been as much subject to the influence of the urari as the left sciatic. Nevertheless, while the trunk of the left sciatic seems to have entirely lost its irritability, that of the right seems to have suffered very little indeed. The difference really is, that the left sciatic trunk cannot manifest its irritability because all its branches are poisoned; the right sciatic can, by means of those branches which through the ligature have been removed from the influence of the poison-bearing blood.

With moderate doses of urari, the small branches appear to be poisoned and to have lost their irritability, while the trunks are still intact.

Obs. IV. In a fresh, strong frog, dissect out a gastrocnemius (or any other single muscle), dividing both insertion and origin and ligaturing its blood vessels, thus leaving it connected with the rest of the body by its nerve only. Poison the frog with urari.

It will be found that stimulation of the nerve fibres supplying the muscle at any part of their course, whether close to the muscle, or in the sciatic trunk as far away as possible from the muscle, will produce contractions in the muscle, though all the other motor nerves in the body seem to have lost their irritability.

In a similar way it may be proved that if only the portion of nerve immediately next to the muscle be kept from the influence of the poison, however much the rest may have been subjected to the action of the poison, the muscle may be thrown into contractions by stimuli applied to any part of the course of the nerve. The presumption is, that *urari acts on the extreme ends only of the nerve,* possibly on the end-plates.

Yet, as we have seen, however much the muscles themselves be exposed to the action of the poison, they do not lose their irritability. These two facts (1), that urari poisons the extreme peripheral ends of the nerves, and (2), that the muscles themselves do not under urari lose their irritability, form together a very strong argument for the view that muscles possess an independent irritability of their own.

Obs. V. Get ready a nerve-muscle preparation. Place one pair of electrodes (A) (as far apart as practicable) on the muscle itself, another (B) on the nerve near the muscle, and a third (non-polarizable) pair (C) on the nerve also, a little higher up than B. Connect A and B with induction coils, and determine the minimum stimulus required to be sent through each pair of electrodes in order to produce a contraction in the muscle. It will be as well to record the contraction by means of the lever, &c. The irritability of the nerve (electrodes B) and of the muscle and nerve together (electrodes A) will thus be respectively determined.

Now pass through C a strong ascending constant current; and while the current is passing, determine as before the minimum stimulus for A and B. By the ascending constant current the portion of nerve between the electrodes C and the muscle has been thrown into a state of anelectrotonus; and it will be found that the irritability of the nerve in this region has been very considerably lowered; or, if the polarizing current be strong enough, and the pair of polarizing electrodes far enough apart, has been suspended altogether. Contractions in the muscle are either entirely absent when a shock is sent through B, or only appear when the shock is very strong. At the same time it will be found that the minimum stimulus of A is not very different from what it was before. A rather stronger stimulus is required to produce a contraction, but the difference is strikingly less than that in the case of the electrodes B, and even this difference may be accounted for by considering that the electrodes A stimulate both the muscular fibres and the intra-muscular nerve fibres, and that the combined effect is therefore greater when the intra-muscular nerves are intact than when they are paralyzed by the ascending current.

Thus the ascending current will, if strong enough, suspend the irritability of the nerve fibres supplying a muscle, and yet will leave the muscle but little altered in its susceptibility to direct stimulation. This again is an argument in favour of "independent muscular irritability."

The same view is supported by the facts that the chemical irritants of nerve and muscle are not identical (*see* Chapter XXX., *Obs.* V.—VII.); that the lower part of the sartorius of young frogs in which no nerve fibres can be detected, is susceptible of chemical stimulation; and that the idio-muscular contraction may be called forth in muscles the nerves of which have completely lost their irritability. (Chapter XXX., *Obs.* IV.)

CHAPTER XXXII.

THE FUNCTIONS OF THE ROOTS OF SPINAL NERVES.

THE *posterior* root of a spinal nerve is said to be *sensory, i.e.,* to serve as the path along which alone *centripetal* influences pass on their way from the peripheral nerve terminations to those central organs, in which they become transformed into sensations, or give rise to reflex actions, &c. The *anterior* root is said to be *motor, i.e.,* to serve as the path along which alone *centrifugal* impulses pass, on their way from the central organs to the nerve terminations in muscles, &c. The truth of this absolute distinction in function between the two roots may readily be shown in the frog.

The results are most clear and distinct when the organs of consciousness are intact, and the ordinary tokens of sensation are used to determine whether the impulses caused by stimulation of the peripheral terminations reach the conscious central nervous system or not. But the facts may also be readily shown in the absence of the brain, when reflex action is taken as a proof of a centripetal impulse having reached the spinal cord. In the former case, the frog should be placed under chloroform during the laying bare of the roots. In the latter, the medulla should be previously divided in the neck (*see* Chap. XXXIII.).

The frog being placed on its belly, make an incision in the middle line of the back, from the upper end of coccyx to the level of the fore limbs, *see* fig. 266 *g h*. Having hooked back the flaps of skin, carry the median incision down to the spines of the vertebræ, and dissect away the longitudinal muscles on either side, so as to lay bare the bony arches, and then hook back the muscles on either side, or cut them away altogether.

With a small but strong blunt-pointed pair of scissors, cut through, on either side, the arch of the last (eighth) vertebra (be careful not to thrust the scissors in too deep), and remove the piece so loosened. Proceed then to the next arch above, and so remove three arches. The roots of the nerves will be seen lying in the spinal canal. Snip away the remains of the arches on each side, until the last three (or four) roots are quite clear, being very careful not to touch the nerves with the scissors. The bleeding may be disregarded. The posterior roots lie superficially, are large, and hide the anterior roots. The several roots may be separated from each other by passing with great care the blunt seeker lengthways between them.

Very gently pass a fine aneurism needle, armed with thin silk, (ex. gr., a fine sewing-needle, with the head slightly bent, and the point fixed in a handle), under a conspicuous posterior root which seems to be the last. This will be the ninth; the tenth is much smaller, and runs closer to the filum terminale, *see* fig. 295. The seventh, eighth, and ninth form the ischiatic, from which the crural, *Nc*, and sciatic, *Ni*, nerves are given off, the seventh supplying most of the fibres of the crural. Tie the silk loosely round the nerve, near its entrance into the cord. Carefully avoid compressing the nerve.

Obs. I. The frog being completely at rest, draw the ligature tight, observing the frog all the while. If the animal be in good condition, some movements will be visible in some parts of the body as evidence either of sensibility or reflex action. Now cut the nerve between the ligature and the cord; some movement will probably be again witnessed.

Obs. II. Lift the *peripheral* stump of the nerve carefully up by means of the ligature, and slip it upon the curved shielded electrodes (fig. 271) which may be held in the hand, or, better, fixed on a movable stand. To prevent any escape of the current, slip a fragment of india-rubber sheeting beneath the nerve and electrodes, so as to isolate these from the cord and from the rest of the nerves. Pass a moderately strong interrupted current, through the electrodes. If there be no escape of the current the animal will not move in the slightest.

Obs. III. Repeat the observation with the nerve-root next above (the 8th), with this difference; place the ligature as near as possible to the walls of the spinal canal; divide the nerve between the ligature and the wall, and place the *central* instead of the *peripheral* stump on the electrodes.

Ligature and section, as before, produce movements. A very moderate interrupted current applied to the *central* stump will produce very considerable movements in various parts of the body, *i.e.*, signs of sensation or reflex action, as the case may be.

Ligature or section of the posterior roots of spinal nerves produces movements in various parts of the body. Stimulation of the peripheral stump produces no movement whatever; stimulation of the central stump produces considerable movements. These movements, be they simple reflex actions or more complicated voluntary movements set going by conscious sensations, are evidences of centripetal sensor impulses, excited in the posterior sensory roots.

Obs. IV. Examine now the sensibility of the hind limb on which you have been operating. It will be found that pinching the toes or the skin of the hind surfaces of the limb produces little or no reflex action. The anterior surface of the leg, however, still retains considerable sensibility.

Obs. V. Divide the posterior roots of the 7th and 8th nerves, and also that of the small 10th nerve. The whole limb will now be found to be totally insensible. Movements of the leg, however, may readily be called forth by pinching the skin of the back, or any other part of the body except the leg itself.

Division of the posterior roots stops the passage of sensory, but not of motor impulses.

Obs. VI. Carefully cut away the posterior roots on which you have been experimenting. The anterior roots, which are thinner than the posterior, will now come into view.

Repeat on one of these anterior roots (9th nerve) *Obs.* II. Mere touching the nerve will probably produce a movement of the hind limb of that side. This result will at all events follow upon ligature and section.

Stimulation of the peripheral stump, with even a very feeble stimulus, will produce tetanus in the limb.

Obs. VII. Repeat on the anterior root next above *Obs.* III.

No effect whatever will be produced by stimulating the central stump.

The anterior roots convey motor impulses centrifugally, but not sensory impulses centripetally.

Obs. VIII. In a fresh, strong frog lay bare the roots of the spinal nerves and divide the posterior roots of the 7th, 8th, 9th, 10th nerves on the right side and the corresponding anterior roots on the left side.

The left leg will remain motionless, being simply dragged along by the rest of the body, but never moving of itself. [If the brain has been previously destroyed or separated from the spinal cord, the right leg will be drawn up as usual (*see* Chap. XXXIII.), but not the left leg.]

Pinching the right foot, or otherwise irritating the right leg, will give rise to no movement whatever in any part of the body, will call forth no signs of sensation.

Pinching the left foot, or otherwise irritating the left leg, or any part of the body except the right leg, will produce movements which may occur in any part of the body except the left leg itself.

In this case the right leg has had all its posterior, the left all its anterior, roots divided. No centripetal impulses pass up from the right leg to the central nervous system; no centrifugal impulses pass down from the central nervous system to muscles of the left leg.

The posterior roots are the channels of the centripetal (sensory), the anterior of centrifugal (motor) impulses.

Recurrent Sensibility.—This is never witnessed in the frog. It can only be shown in the higher animals, the cat or dog being best adapted for the purpose. The method adopted is very similar to the above—the arches of one or two vertebræ being carefully sawn through or cut through with the bone forceps, and the exposed roots being very carefully freed from the connective tissue surrounding them. If the animal be strong and have thoroughly recovered from the chloroform and from the operation, irritation of the *peripheral* stump of the anterior root causes not only contractions in the muscles supplied by the nerve, but also movements in other parts of the body indicative of pain or of sensations. On dividing the mixed trunk at some little distance from the junction of the roots, the contractions of the muscles supplied by the nerve cease, but the general signs of pain or of sensation still remain. These disappear when the posterior root is also divided. Hence it is inferred that fibres conveying centripetal impulses pass downward along the anterior root to the mixed trunk, and thence, turning round, run back again to the central nervous organ along the posterior root. (For further details, *see* Bernard, Leçons sur la Phys. du Système Nerveux, Vol. I., p. 62 *et seq.*)

CHAPTER XXXIII.

REFLEX ACTIONS.

Reflex actions are best studied in the frog, the brain having first been removed, or at least separated from the spinal cord. The strongest and healthiest frogs should be chosen for the purpose. The student should make himself acquainted with the general form of the dried frog's skull. This having been done, the position of the occipito-atlantal articulation may readily be recognized on the living animal.

Division of the Medulla Oblongata.—Having wrapped a cloth round the hind legs and body of the animal, clasp the fore legs round the ring-finger of the left hand, and hold them in position by the middle and little fingers, which should also hold tight the cloth. Press down the tip of the frog's nose with the thumb of the same hand, so as to bend the neck as much as possible. If the fore-finger of the right hand be now made to glide over the roof of the skull, exactly in the mid-line from before backwards, a slight but distinct depression will be felt in the neck at the point where the occiput ends, and where the medulla is covered, not by bone, but by the occipito-atlantal membrane. It lies in a line drawn across the skull at a tangent to the hinder borders of the two membrana tympani. (Fig. 266, line *a-b.*)

The position of this point being satisfactorily ascertained, with a sharp-pointed scalpel make a small transverse incision across it about a few millimetres long. The incision should not be carried too far on either side. If the blood, which comes freely, be rapidly taken up with a sponge, and the neck be kept well bent, the medulla will be clearly seen. This should now be completely cut across, and the wound be rapidly sponged, in order that the division may be ascertained by actual inspection to be complete. The encephalon may then be completely destroyed by introducing a blunt piece of wire into the wound, and eviscerating the skull. If the wound be then left to itself, the bleeding will, in most cases, soon cease; if not, a small plug of wood (the sharpened end of a lucifer-match) may be thrust into the skull. This, however, should be avoided if possible. It is better to conduct the operation in this way, seeing clearly what is being done, than to divide skin, membrane, and medulla by one thrust, without being able to tell exactly whether the division is complete.

Decapitation.—Introduce one blade of a strong pair of scissors into the mouth, and bring it, transverse to the long axis of the head, as far back as possible. Bring the other blade down to the skin behind the occiput, and quickly cut off the head, being careful that neither blade slips forward. Simple inspection will at once determine whether the whole of the encephalon has been removed or no. The bleeding in many cases is excessive, and must be staunched by astringents or by the actual cautery. Indeed, where decapitation seems desirable, it is far better to employ the galvanic cauture, introducing the loop of platinum wire into the mouth, and bringing it out through the occiput along the line *a-b*, fig. 266.

For the general study of reflex actions, division of the medulla is preferable to decapitation. The large amount of bleeding, the exposure to the air, and possibly other causes, often lead, in the latter case, to abnormal results, *ex gr.*, pseudo-voluntary movements on the one hand, and lack of reaction on the other.

Obs. I. Place the frog, immediately after the division of the medulla, on its belly, with its legs extended. In most cases the legs will remain extended, and at first no movements will be produced by stimuli applied to any part of the body. The animal (or rather its spinal cord) is in a state of shock, consequent upon the operation.

If the animal be watched, it will be found that after a while the hind legs, apparently without the intervention of any external stimulus, are suddenly, first one and then the other, drawn up to the body, and assume the wonted flexed posture. This is a token that the condition of shock has passed away. If now one of the legs be pulled out, and then let go again, it will be immediately drawn up once more under the body.

After the shock has passed away, the legs having been drawn up, the animal will appear to have assumed a natural posture. On observing it more closely, however, it will be found that the posture is not quite natural. The line of the back is too horizontal, the head lies flat, with the neck almost touching the table, and the fore limbs spread out; whereas an entire frog keeps the head and neck raised high up on the almost vertical fore limbs, and the line of the body makes a large angle with the plane of the table.

If left to itself, the frog will exhibit no movements whatever, will not stir from the spot in which it is placed unless some external stimulus be brought to bear upon it. This absence of spontaneous movements is most marked, when sudden variations of temperature are avoided, and the skin is not allowed to get dry. Hence it is advisable to place the animal on a dish containing a small quantity of water, and to cover it with a glass shade.

If turned over and placed on its back, it remains for an indefinite period in that position, without making any attempt to regain its natural posture. While on its back, the heart may be observed beating, but the respiratory movements will be wholly absent.

If thrown into a basin of water, it will sink to the bottom like a lump of lead (unless the lungs be too much distended with air), without making any attempt whatever to swim.

By irritating it in various ways, it may be made to execute a variety of movements (*see* following observations), but cannot by any means be made to leap or spring forward.

Obs. II. With the point of a needle gently stroke the abdominal walls on one side. A slight twitching of the muscles of the region so stroked will be witnessed. This is one of the simplest forms of reflex action. Contraction takes place in muscles on that side of the body only, the afferent nerves of which are affected by the stimulus, and it will be found that the afferent and efferent nerves concerned in the action belong tolerably exactly to the same segment of the spinal cord.

On increasing the stimulus gradually by stroking more forcibly, the twitchings will be seen to spread over a wider and wider area, to invade the other side, and finally to pass into the hinder and fore limbs.

With a stimulus, sufficiently slight, of an afferent nerve, a definite small group of efferent fibres are alone affected by reflex action. On increasing the intensity of the stimulus, the effect spreads into a larger and larger number of efferent fibres.

Obs. III. Pass an S hook through the lower jaw, and thus suspend the animal on a suitable upright, with the legs and body hanging freely down.

Or, take a piece of thin wood, about an inch broad and five long; place the frog belly downwards on it, in such a way that the wood reaches no farther down than the lower part of the abdomen, and secure the frog to it by two slight india-rubber bands, one immediately below the fore limbs, and the other a little above the thighs. If the wooden slip be now fastened vertically in an upright, the hind limbs will hang freely down, completely loose, while the body will be held sufficiently firm.

For most purposes the former simpler method is sufficient. When it is desired to study the movements of the legs alone with some accuracy, the latter method must be adopted.

The legs hanging freely down, and the body being completely at rest, with a smooth pair of forceps gently pinch the tip of one of the toes. The leg will immediately be drawn sharply up, and after being kept in the flexed position for a variable time, will be slowly dropped again.

Repeat the observation on the other leg. Only that leg, the toes of which are pinched, is drawn up; and if the toes be not too roughly treated, no other movement than the drawing up of the leg is witnessed.

Obs. IV. With more force pinch the folds of skin around the anus. Both legs will be suddenly completely drawn up, so that the toes of both feet are brought above the forceps, and are then as suddenly and completely extended again. This movement of sudden flexion and extension, that is of kicking, may be repeated rapidly several times as the result of one forcible pinching of the region in question.

Obs. V. Pinch with some force the skin at a point on one side of the loins. The leg of the same side will be suddenly flexed over the back, and brought round back again with a sweeping movement.

Obs. VI. The hind limbs hanging down as before, place a watch or other small glass containing very dilute sulphuric acid (one drop to 20, 30, or 50 CC^{m}, strong enough to give an acid taste) underneath the frog, and bring it close up to one of the feet, so that the extreme tip of the longest toe just dips into the acid. Within a short time, the exact length of time being determined by the strength of the acid and the condition of the frog, the leg will be flexed, and the foot withdrawn. Very frequently the movement, even after the fluid has been taken quite out of the way, is not confined to a single flexion followed by a relaxation, but consists of a series of flexions and relaxations, each succeeding flexion being less marked than its predecessor.

Repeat the observation with varying degrees of acidity, beginning with simple distilled water, and gradually adding acid. Be careful to wash the foot carefully with water after each observation, to wait some minutes between each application, and to dip only the tip of the toe, and that to the same extent in each case.

Measure by means of a metronome, beating very rapidly, the exact time intervening between the actual entrance of the toe into the fluid, and its withdrawal.

With an acid of a given strength, applied to the same frog under varying circumstances, the duration of this interval may be taken as a measure of the power of reflex action. The shorter the interval, the more prone is the cord to reflex actions. In making observations on the length of this interval, it is as well to use very dilute acid, such as will only just give a sensation of acidity when applied to the tongue.

Obs. VII. Simple water of a sufficiently high temperature

(25°—35° C.) may be used instead of the acid. It has the advantage of being less likely than the acid to produce a permanent action on the skin. The difficulty, however, of keeping up exactly the same temperature renders it unsuitable for comparative experiments.

In all the above experiments the movements produced bear marks of purpose. As the result of stimulation of a particular region of the surface of the body, we find a complicated movement, a movement brought about by the contraction of certain muscles and sets of muscles, acting in a definite combination and sequence. The movement thus produced is apparently directed towards an end. Thus when the foot is pinched or irritated by the acid, the resulting movements appear at least directed towards, and frequently actually effect, the withdrawal of the foot from the offending object; when the flank is pinched, the movement is such as tends to thrust away the points of the forceps; when the anus is pinched to kick away the forceps, and so on.

This purposeful character of reflex actions may be still more conveniently shown by adopting the following method:—

Obs. VIII. Arrange the frog with the legs alone free according to the second method given above. Cut small pieces of blotting-paper about 1 or 2 millimetres square, dip them in strong acetic acid, remove from them all superfluous acid, and then place them on definite regions of the skin. In this way the stimulus may be limited to very small areas chosen at pleasure; and it will be found that very different movements of the hind limbs will be produced by applying the morsel of paper to different regions of the body. Thus, if the morsel be placed on the heel of one foot, both feet will be violently rubbed together, while the legs remain forcibly extended. If the morsel be placed on one flank, it will be rubbed off by the foot of the same side; if it be placed in the mid-line of the back, either or both feet will be employed to remove it, and so on.

The student will do well to map out the limbs and body of the frog into small areas, and to determine the characters of the movements, which result from the stimulation of each area. He will in this way find abundant instances of an apparent purpose.

Obs. IX. It has been seen that where the morsel of acid paper is placed, say on the right flank, it is the right leg, and the right leg only, which under ordinary circumstances is used to rub off the paper. Choosing a strong frog, in which reflex action has been found to be highly developed, suspend it according to the second method, hold the right leg firmly down, or load it with a greater weight than the leg is able to lift, and apply a morsel of acid paper to the right flank. Twitchings and convulsive movements of the right leg are first witnessed, and then the *left* leg is brought up to rub the right flank.

Place a similarly strong frog with powerful reflex capabilities on its back on the table.

If a morsel of paper were now placed on the surface of the right thigh, the right foot would be brought up to rub away the paper, the left foot remaining quiet. Hold tight the right foot, or better still, place a ligature below the right knee, and cut away the whole lower leg and foot. If the acid paper be now placed on the right thigh, convulsive twitching of the stump (ineffectual as far as the removal of the paper is concerned) will follow, and then the *left* foot will be brought across to rub the paper away.

In both these cases we have instances of an apparent power of the organism, even in the total absence of the brain, to change its customary proceeding and to adapt itself at once to new circumstances, instances which have led some to speak of a conscious intelligence residing in the spinal cord.

Obs. X. As an instance tending directly to the contrary supposition, the following experiment may be performed :—

In a shallow glass or porcelain dish, place enough water to reach up to the head of a frog. Line the sides and bottom of the vessel inside with felt or blotting-paper.

Place an unmutilated frog in the water, and then gradually raise the temperature. Cover the top of the vessel with a piece of gauze or netting, to prevent the escape of the frog.

As the temperature rises the frog becomes uneasy, and after 20° C or 30° C is reached makes violent attempts to escape.

Place in exactly similar circumstances a frog whose medulla has been divided; the water should cover the whole of the animal up to just below the wound in the neck (care being taken that the water gains no access to the spinal cord).

Up to 30° or above, no movement of any kind is visible. About 35°, slight twitchings may be observed in some of the muscles of the limbs and flanks. At 38°—40° the whole body becomes rigid (rigor caloris), and the frog is dead without having made the slightest attempt to escape from the hot water.

This observation goes quite as far to prove that the frog, in the absence of the brain, has no consciousness or volition as Observation IX. seems to point to the contrary. Both observations are probably to be explained without any reference, negative or positive, to consciousness or volition.

Obs. XI. As a useful exercise, the student may lay bare the roots of the 7th, 8th, 9th, and 10th spinal nerves as directed in Chap. XXXII., the medulla having previously been divided. Let him now divide the posterior root of say the 7th nerve, and determine on what parts of the skin the acid papers may be placed without producing reflex actions. In this way he may ascertain the distribution in the skin of the sensory filaments of that nerve; and in the same way with the other nerves.

Obs. XII. Having divided the medulla, make a transverse incision over the spine a little below the level of the fore limbs (fig. 266, line *c-d*) cut through very carefully a vertebral arch on each side of the middle line and remove the piece. With a sharp-pointed scalpel, the spinal cord may be divided right across.

After the shock has passed away, it will be found that reflex actions may be called forth in the fore limbs by stimulating the skin of the fore limbs or of the fore part of the body, without any movement whatever being produced in the hind limbs; and vice versâ. By the operation, the body has become divided into two segments, which, as far as all reflex actions are concerned, are quite independent one of the other. Sometimes, when the movements of one segment are very violent, the other segment becomes displaced, the displacement serves as a stimulus, and a reflex action is thereby indirectly brought about. But this will not be confounded with direct reflex actions, which can only be called forth by stimulating the respective segments.

Obs. XIII. In any of the above frogs which have shown good reflex actions, destroy the spinal cord entirely by thrusting a wire or blunt needle down the spinal canal. All reflex actions will at once and for ever cease.

Obs. XIV. The orderly and purposeful character of reflex actions may be modified by the action of certain poisons, more particularly by strychnia.

Having divided the medulla in a frog, suspend the animal as in *Obs.* III. and determine the readiness with which reflex action is produced by mechanical stimulation. This may be taken as a measure of the reflex excitability of the spinal cord (the acid method being unsuitable in this case).

Introduce into the back of the frog a $\frac{1}{2000}$ or $\frac{1}{3000}$ of a grain of strychnia sulphate and determine again after a short interval the effects of mechanical stimulation. They will be found to be increased, *i.e.*, the reflex excitability has become heightened.

Now inject a larger quantity of the poison, and in a very short time a very marked change becomes obvious. The movement resulting from the stimulus is no longer a simple movement, for instance, a simple withdrawal of the foot, but a tetanic extension of the leg, which becomes more and more violent and prolonged.

Soon each application of the stimulus will give rise to a prolonged tetanic movement which is no longer confined to the limb, or even to the side stimulated. The hind limbs are forcibly extended, the fore limbs bent over the sternum, and every muscle of the trunk is thrown into a state of prolonged tetanic contraction.

After a while these contractions pass off, and the body and limbs become once more relaxed. With each application of the stimulus the same *tetanus* of the whole body is called forth, no matter to what part of the body the stimulus be applied, or what be the character of the stimulus. The purposeful normal reflex actions are lost in a complete spasm of the whole body.

It is possible to conceive that this result might be brought out by an abnormal intensity of the impulses generated in the afferent nerve by the stimulus, or by an abnormal irritability of the total muscular system, or by an abnormal condition of the spinal cord. That the last and not either of the former two is the real cause is shown by the following observation.

Obs. XV. In a frog with divided medulla, ligature the hind limbs, leaving the nerves free as directed in Chap. XXXI. for urari, and afterwards inject a small dose of strychnia.

In spite of the absence of the blood-current in the lower limbs, the reflex actions will be as manifest in them, and as easily brought about by stimulating them, as under ordinary circumstances. But by the ligature the strychnia has been prevented from having access to either the sensory nerves or the motor nerves and muscles of the hind limb. Hence the tetanic character of the reflex actions produced in them must be due entirely to the changed conditions of the spinal cord itself.

CHAPTER XXXIV.

ON SOME FUNCTIONS OF CERTAIN PARTS OF THE ENCEPHALON.

Most of the experiments illustrating this part of the subject, like those having to do with the conduction of impulses through the spinal cord, are of a kind which the student cannot be expected to perform for himself, and are consequently not introduced here. Several observations, however, of a very instructive character may be made on the frog.

The brain of the frog may be considered, for present physiological purposes, as consisting of three segments. We have first the medulla oblongata (fig. 296 M. O), and small cerebellum c, next the optic lobes, L. Op., easily recognized in an operation by the pigment contained in their pia mater, and lastly, the cerebral hemispheres H. C lying over the corpora striata, with the small optic thalami Th. O between them and the optic lobes.

The position of the optic lobes pretty well corresponds to the hind part of the fronto-parietal bones, which are distinctly seen when the skin over the skull is removed. A transverse incision made through the skull with a narrow strong blade, in a line which runs as a tangent to the anterior borders of the membranæ tympani, will separate the cerebral from the optic lobes. This may be done without removing even the skin. In most cases, however, it is better to remove the roof of the skull and to see the parts of the brain which are being operated on.

The frog being placed under chloroform, make a longitudinal incision over the mid-line of the skull from behind the nose backwards, and convert it into a T incision by a transverse cut immediately behind the membranæ tympani (fig. 266, *e. f a b.*). Hook back the flaps. With a pair of fine bone forceps or strong scissors cut right across the fronto-parietal bones where they overlap the ethmoid. Each bone may then be easily seized by its front end and torn away without any injury to the cerebrum below. That being done, the blade of a pair of scissors may be carefully slipped under each parietal bone close to its external border and the bone cut through. The bones may then be carefully seized at their front border with a pair of forceps, lifted up and torn away. If the blood vessels at the side have been avoided, there will be but little bleeding, and what does occur will soon cease. The cerebrum may now be simply divided from the optic lobes by a transverse incision and removed. A better method, in order to prevent any injury to the optic nerves and optic thalami, is to cut across the cerebral lobes at their junction with the olfactory lobes, fig. 296 L. ol., to lift up their cut ends and so to remove them carefully, working gradually backwards. To separate the optic lobes from the medulla, nothing more than a simple transverse incision is necessary, taking care not to injure the cerebellum; but it is as well to remove all the parts in front of the incision. The flaps of skin may then be brought together and united by a couple of sutures, and the animal left to recover from the operation. All plugging, &c., should be avoided.

Obs. 1. *The phenomena of a frog when the animal possesses the medulla oblongata and cerebellum as well as the spinal cord, but all the rest of brain has been removed.* The following facts may be observed after the animal has completely recovered from the operation, and should be compared with the phenomena of a frog possessing a spinal cord only.

The attitude is completely normal, quite different from that of a frog possessing the spinal cord only. The head is well raised on the fore limbs.

Respiration goes on in an almost normal manner.

If left to itself, and protected from all external stimuli, the animal will remain perfectly motionless. For some little time after the operation has been performed, movements apparently voluntary, that is, occurring without any obvious cause, are frequently witnessed. These, however, generally cease after a little while, and if the animal lives long enough for the wound to heal, entirely disappear.

The animal will not feed of itself. Flies, worms, &c., may be placed close to it, and even introduced between the teeth, without any notice being taken of them. If, however, the mouth be opened and a morsel be introduced into the pharynx, it is swallowed. In this way the animal may be kept alive for an indefinite period, being fed on pieces of worm or flesh; frog's flesh does very well; care must be taken not to introduce too large pieces, and not to feed too often.

If the skin round the anus be pinched, the animal does more than simply kick out its hind legs: it leaps forward, often repeating the leap several times, and springing forward a considerable distance; sometimes it crawls instead of leaping, and not unfrequently does both. If placed on its back, it immediately turns over again to its normal position. This it does instantly and with vigour. It has to be held down forcibly in order to keep it on its back for any length of time.

If thrown into a basin of water, it at once begins to swim, and continues swimming about with considerable energy till it finds some resting-place. Having found a suitable support, it crawls upon it, and assumes the normal attitude, and there remains motionless until again disturbed.

If the cerebellum be removed, all these movements and habits become much impaired, much feebler, and less striking; or may (with the exception of the respiratory movements) be wholly absent, but it is difficult to remove the entire cerebellum without injury to the medulla. Hence the share taken by each organ in keeping up these powers of executing complicated movements cannot be readily ascertained.

The above facts all point to the existence in this part of the brain of some mechanism connected with the *co-ordination of movements*. The crawling, leaping, swimming, and turning over on to the belly all demand a more complex nervous machinery than is needed for the purely spinal reflex actions, intricate as many of these are.

The persistence of what we have called the normal attitude is very remarkable. Strictly speaking, the natural frog varies its attitude constantly, but its most common posture, the one into which it naturally falls when at rest, is the one we have described. This attitude is the one to which the frog with cerebellum and medulla clings most rigidly, to which it always returns after being disturbed, and in which it eventually dies if left alone and not fed.

Obs. II. *Influence of the presence of the optic lobes.*—Remove the parts in front of the optic lobes as directed; the best results are obtained when the animal is allowed to remain perfectly quiet for a day, or for several hours at least, after the operation.

All the facts mentioned in *Obs.* I. may also be observed in this case; in addition, there are certain phenomena which are only witnessed when the optic lobes are present.

Goltz's Balancing Experiment.—Place the frog on a rough board (about eight or nine inches square), somewhat near to one of the edges. Hold the board horizontal, and the frog will remain motionless in the normal attitude.

Tilt the board gradually up, with that edge uppermost which is farthest away from the frog, and towards which he should be looking. Up to an angle of about 45° and beyond no change will be observed in the frog. As soon, however, as the board becomes so much inclined that the centre of gravity of the frog is thrown outside the lower edge, the frog will begin to creep up the board. As the inclination proceeds, the frog moves higher and higher up, until, when the board at last becomes vertical, the frog will be found seated in the normal attitude on the upper edge. On continuing the movement of the board, so that what was the upper surface becomes the lower, the frog will move from the edge downward over the now upper surface; and when that surface, by the continuance of the revolving motion, again becomes inclined upward, will again creep over it as before towards the new upper edge.

Evidently here the disturbance of the centre of gravity produces such an effect as to give rise to movements which are directed towards the re-establishment of equilibrium, and which are continued until that result is achieved. At first sight this may appear very much like an act of conscious intelligence, but if the student carefully observes the different behaviour of an entire frog and of a frog in this condition, the contrast between the two will be found very striking. This frog does nothing but crawl, and stops crawling as soon as the stimulus of the disturbed equilibrium passes away. When the experiment is successful, he remains perched motionless on the edge of the vertical board, and never leaps away. The entire frog leaps away at once.

Goltz's Croaking Experiment.—Place the frog on the table, and with the thumb and fore-finger gently stroke down the flanks on either side. A little very gentle pressure must be exercised. As the thumb is thus carried backward along the sides of the animal, he will utter a single distinct sharp short croak, and as often as the movement is repeated the croak will be heard.

This again is very different from the behaviour of the entire frog. The entire frog, when thus stroked, may or may not croak; for a single stroke he may croak several times, or not at all. The frog without the cerebral hemispheres, but possessing the optic lobes, and otherwise in good condition, croaks at every stroke, and croaks once only for each stroke.

One seems driven to regard this behaviour as the result of a, so to speak, croaking mechanism; and not as the act of a conscious intelligence.

Obs. III. The cerebral hemispheres having been carefully removed, in such a way as to leave intact the optic nerves, the student may attempt the following experiment of Goltz to test the persistence of any visual sensations.

Place the frog on the table, with his head towards the window, and some six or eight inches in front of him place a large book, or other thoroughly opaque mass. Gently pinch him behind, in any spot which is exactly in the median line of his body. Under ordinary circumstances, he would spring forward in a straight line, and, in the absence of all vision, would strike his head against the book. It will be found in this case, however, if the experiment be successful, that instead of springing forward in a straight line, he turns a little to the right or to the left, so as to avoid the book.

If he turns to the left, shift the book to the left and then repeat the experiment. He will now move in a straight line or to the right. In the same way, if the book be to the right he will incline to the left.

The student will do well to try this experiment, but it frequently fails. Care should be taken to have the light coming into the room as directly in front of the animal as possible, in order to exaggerate the shadow cast by the book. Apparently the image of the opaque book produces some sort of visual impression sufficient to guide the movements of the animal. But it would be hazardous to say that the animal sees, for it is difficult, or rather impossible, to obtain any other evidence of the influence of vision in a frog in such a condition.

These observations are introduced to illustrate the fact that, in the absence of the cerebral hemispheres, whether the optic lobes be present or no, the frog possesses no volition. He executes none of those so-called spontaneous movements which we are in the habit of attributing to volition. This leads us to infer the absence of at least that amount of consciousness which we find inseparably connected with volition. At the same time, we learn that the presence of certain parts of the brain lying behind the cerebrum, determines the nature of the movements which are called forth by external stimuli, rendering them very complicated and delicate, especially giving them features which cause them closely to resemble ordinary voluntary movements, and suggesting the idea of intricate arrangements within certain parts of the brain, of afferent (including nerves from the sense organs) and efferent nerves and nervous centres, which may be set into action by volition on the one hand, or by some external stimulus on the other.

Obs. IV. *Inhibitory Influence of parts of the Brain over the Reflex Actions of the Spinal Cord.*

The reflex actions of the cord much more readily occur, and are much more vigorous and complete, in the absence than in the presence of the brain. The brain, therefore, must in some way or other prevent reflex actions.

Irritation of the optic lobes.— Having prepared a frog, as in *Obs.* II. &c., ascertain the intensity of the reflex activity by the sulphuric acid method. (Chapter XXXIII., *Obs.* VI.)

Touch with a small crystal of sodium of chloride, or with the point of a brush dipped in saline solution, the cut surface of the optic lobes and determine, after a few seconds before convulsions, which may occur, have set in, the duration of the interval between the exposure of the foot to the acid and its withdrawal. It will be found to be greatly prolonged. In other words, irritation of the optic lobes has interfered with, or partially inhibited, the reflex action of the cord. If the optic lobes be removed, and the medulla irritated instead, the result will be much less marked.

Obs. V. Having prepared a frog with optic lobes, and determined the reflex interval as above, inject into the back $\frac{1}{4}$ grain of quinine sulphate and determine the interval again from time to time. It will be found to be much prolonged.

Having prepared a frog with divided medulla (Chapter XXXIII.), and determined the duration of the reflex interval, inject the same quantity of quinine, and again determine the interval as before. No prolongation of the interval will be observed. These results may be explained by supposing that the quinine is unable to act directly on the reflex activity of the cord, but is able either to stimulate an inhibitory mechanism in the brain, or at least to affect the brain in such a manner as to interfere with the reflex actions of the chord.

Obs. VI. *Removal of the Cerebral Hemispheres in the Bird.*—Select a vigorous pigeon, so young as to be just able to fly well. Keep it on dry food for a few days, in order to avoid an excess of bleeding.

Having placed it under chloroform, using as little chloroform as possible, make an incision in the median line over the roof of the skull, and hook back the two flaps of skin. The thin skull may now be easily cut through with a pair of scissors, and the roof removed. Without waiting to stop the bleeding, draw the cerebral hemispheres gently forward, and carry a traverse incision from side to side with a blunt-pointed bistoury through the brain in front of the *corpora bigemina*, and with a narrow spatula remove the hemispheres *en masse* from behind forwards. Place the animal on a perch, and leave it to itself. Do not attempt to plug or staunch the bleeding; a clot will soon form and serve as the best protection against further bleeding. Postpone putting sutures into the flaps of skin until the bleeding has wholly ceased.

If it be desired to keep the animal alive for any length of time, it will be as well to allow it to remain perfectly quiet for some time after the operation, avoiding all observations and experiments upon it. Only on the second or third day begin to feed it gradually with a few grains of softened barley or of rice.

Otherwise, observations may be begun as soon as the bleeding has ceased.

The bird so deprived of its cerebral hemispheres (together with its *corpora striata* and *optic thalami*) if placed on the finger or on a perch, will settle itself in a balanced position, and remain thus for an indefinite period motionless, or all but motionless, except as far as the breathing is concerned. It seems to be plunged in the most profound sleep, with the head drooping and the eyelids closed.

If irritated, it appears to awake; it opens its eyes, raises its head, and more or less opens its wings, and otherwise moves its body or limbs.

If, while in a state of complete rest, perched on the fore-finger, the finger be gently revolved, so as to throw the centre of gravity outside the finger, the wings will immediately spread out as if for the act of flight.

If thrown into the air, it will actually fly for some little distance, eventually settling down into its lethargic but balanced condition. If in its flight it meets any objects it blindly strikes against them.

For a detailed description of the phenomena exhibited by such a bird, *see* Flourens' Système Nerveux, p. 123.

Obs. VII. *Removal of the Cerebral Hemispheres in the Mammal.*—A young rabbit, about two months old, is the most suitable animal to operate upon. It should be fed for some days previously on dry food. The method of operating is very much the same as in the bird. Fasten the animal on a Cermak's rabbit-holder (fig. 204), which should be raised at an angle of 60° or so, in order that the head may be as high as possible, and, consequently, the bleeding diminished. The removal of the roof of the skull will be facilitated by first making a small hole in each parietal with a trephine about a third of an inch in diameter, and then slipping the blade of the scissors from one hole to the other between the bone and the *dura mater,* and cutting the bone through. The rest of the roof may then be removed piecemeal. Carefully avoid wounding the venous sinuses, and carry the operation through as speedily as possible. The amount of ether or chloroform given should be no more than is absolutely necessary just to send the animal off. Previous ligature of the carotid does very little good, and only complicates the operation.

The animal will not survive the operation very long, but for several hours after the operation the phenomena of complicated movements consequent on stimulation, with total absence of volition, may be witnessed as in the bird and in the frog.

Obs. VIII. *Division of the Semi-circular Canals.*— This is best performed on the bird, *ex. gr.*, a young pigeon. The student should first make himself acquainted with the position and relation of the canals in a dead bird. Make a vertical incision along the back of head, hook back the flaps of skin, scrape away the insertion of the muscles of the neck, remove the outer tablet of the diploe of the skull behind each ear, and pick away in minute pieces with a small pair of forceps the cancellous bone, embedded in which the hard bony canals will then easily be found.

Having thus determined their exact position in the dead bird, the student will find no great difficulty in reaching them by a similar proceeding in the living body. Having found them, cut one or, better still, two on each side right through with a pair of small but strong scissors. The bleeding, which is generally excessive, may be staunched by styptics.

Immediately after the operation, and for an indefinite time afterwards, the bird exhibits the utmost disorder in its movements. Though able apparently to move each and every muscle of its body, it has completely lost the so-called co-ordinating power. For a particular account of this condition, *see* Flourens' Système Nerveux, p. 454, and Goltz Pflüger's Archiv. Vol. III. p. 172.

PHYSIOLOGY.

PART III.—DIGESTION AND SECRETION,

WITH INTRODUCTORY CHAPTERS ON THE ALBUMINOUS COMPOUNDS, AND ON THE CHEMISTRY OF THE TISSUES.

By Dr. LAUDER BRUNTON.

CHAPTER XXXV.

ALBUMINOUS COMPOUNDS.

Section I. — Properties of Albumin.

1. Albuminous bodies occur in all the tissues of the higher animals, and form the chief part of their bulk. They derive their name from white of egg, which may be taken as a type of the group, and they all resemble one another very closely, both in properties and composition. They contain 52·7–54·5 per cent. of carbon, 6·9–7·3 per cent. hydrogen, 20·9–23·5 per cent. oxygen, 15·4–16·5 per cent. nitrogen, and 0·8–1·6 sulphur. In the body they occur partly in a solid form and partly in solution. The herbivora derive them from vegetables in which they are contained, and the carnivora from the animals on which they feed. They do not diffuse, and only a small part of the albuminous matter taken as food passes through the walls of the alimentary canal into the circulation unchanged. The greater portion is converted into diffusible bodies nearly allied to albumin, called peptones, which are readily absorbed.

The organism not only possesses the power of transforming albuminous bodies of one kind into those of another, so that, *e.g.*, the casein of milk is converted into the muscles of the sucking infant, but of combining them with other substances, so as to form such compounds as the hæmoglobin of blood, and of altering them in such a way as to obtain from them the so-called albuminoids of which connective and elastic tissue, cartilage, and epithelium are composed.

After serving their purpose in the organism, they are excreted, not, however, in the form of albumin, but in that of urea. It is extremely improbable that they are converted directly into urea, but rather into leucine and tyrosine, uric acid, kreatin and kreatinine, and other substances, from which urea is produced by farther decomposition. Lately, some have seemed inclined to put forth the opinion that peptones, after their absorption, instead of being raised again to the rank of albuminous bodies, undergo still farther decomposition, and yield hydro-carbons, which serve as fuel to the body, and nitrogenous substances, which are rapidly converted into urea and excreted, while the waste of the tissues proper is supplied by albumin absorbed as such from the alimentary canal (Fick).

** 2. **Preparation of a Solution of Albumin to be used in testing.—Egg Albumin.**—In order to get a solution of albumin for examination, pour the whites of two or three hens' eggs into a beaker, and cut them up with a pair of scissors so as to liberate the albumin from the network of fine membranes in which it is enclosed; stir the viscous fluid vigorously with a glass rod, and then press it through a piece of linen. Mix it with an equal quantity of water, allow it to stand at rest for some time, and then filter it. It passes very slowly through the filter and chokes it very quickly. Several small filters should therefore be used in preference to one or two large ones, and they should be changed as soon as they get choked. The filtration should also be quickened by the use of the air-pump (*see* Appendix, § 211).

This filtrate contains inorganic salts as well as albumin, but it will serve perfectly well to show most of the properties of albumin. For some purposes, however, serum albumin is to be preferred (*see* § 18).

* 3. **Preparation of Pure Albumin.**—If pure albumin is wanted, it may be prepared by separating the inorganic salts from it by dialysis, and this operation is also useful in showing that albumin does not diffuse.

Before subjecting the diluted and filtered albumin to dialysis, it is advisable to concentrate it by evaporation at 40° C., so as to quicken the diffusion of the salts. Then place the concentrated liquid in a dialyser (App. § 212), and let it remain over distilled water. Change the water every six hours, till the water no longer gives a turbidity with silver nitrate. As sodium chloride is the chief salt contained in the egg albumin, its absence in the diffusate may be regarded as a sign that the albumin is free from all salts which will diffuse. The vessels used must be perfectly clean, and the distilled water tested beforehand, as this test is very delicate. The albumin still retains a certain proportion of inorganic salts, but there is no way known of removing them without completely altering its constitution.

4. **Preservation of Albumin.**—If kept in solution, albumin will quickly decompose, and it is inconvenient to prepare it from eggs every time that a solution is required. It may, however, be preserved for a long while by evaporating the solution to dryness at 40° C. (*see* App. § 208). The dry albumin forms a yellowish transparent glassy substance, which may be kept in a stoppered bottle, and dissolved as required.

5. **Serum Albumin.**—Preparation: Add very dilute acetic acid, drop by drop, to serum of blood or hydrocele fluid, stirring it constantly all the time, till a flocculent precipitate is produced. Filter. Add a dilute solution of sodium carbonate to the filtrate till it is nearly neutralized; evaporate it to a small bulk at 40° C.; separate the salts by diffusion, and evaporate the solution at 40° C. to dryness, in the same way as directed for egg albumin. It still contains small quantities of salts, but it is almost impossible to separate them from it.

6. **Differences between Serum Albumin and Egg Albumin.**—Serum albumin agrees with egg albumin in most of its characters, but it differs from it in the following respects:—

1. Its solutions are not coagulated by ether.
2. It is more easily precipitated from its solution by hydrochloric acid.
3. It dissolves more readily in concentrated nitric or hydrochloric acid, and the precipitate thrown down by dilution from the solutions in these acids, as well as that thrown down by these acids from solutions in other menstrua, is readily and completely soluble in the concentrated acids, while the precipitate of egg albumin is not.

When injected under the skin of an animal it does not appear in the urine, while egg albumin does so either when injected under the skin or introduced in large quantities into the stomach or rectum (Stockvis).

* 7. **Solubility of Dry Albumin.**—In testing the solubility of albumin or other substances to be afterwards mentioned, they ought first to be pulverized and then agitated or stirred with the liquid. If the powder runs into masses, these ought to be

broken up with a glass stirring rod; this may be done much more easily if the rod is very thick or has a bulbous end.

If simple agitation or heat suffices to dissolve a substance, it may be placed in a test-tube, but if it requires stirring it should be put in a test-glass (as the rod is apt to break the tube), and afterwards transferred to a tube if heat is to be applied.

The fact of a substance being soluble in a liquid is ascertained by the quantity which was at first put in becoming gradually less, and finally disappearing. When it is only sparingly soluble no diminution in the substance may be observable, and the liquid is then to be decanted or filtered off, and something added or done to it which will indicate the presence of the substance, if any has become dissolved. It is sometimes more convenient, especially when alcohol and ether are employed as solvents, to evaporate the filtered liquid to dryness, and see whether it leaves any residue or not.

Pulverize a little albumin in a Wedgwood mortar. Put a little of it in several test-tubes, and test its solubility in the following reagents :—

†1. Water : The albumin will dissolve, and may be shown to be present in solution by boiling, when it will be precipitated.

†2. Liquor Potassæ : The albumin will dissolve, and may be precipitated from the solution by neutralizing.

3. Alcohol.

4. Ether : The albumin does not dissolve either in alcohol or ether. The clear liquid, when filtered and evaporated, will leave no residue.

†5. Acetic Acid dissolves albumin. On adding potassium ferrocyanide to the solution, a precipitate falls.

†6. Concentrated Hydrochloric Acid : The albumin dissolves, and the solution gradually becomes blue, then violet, and, lastly, brown. Test this with one portion at the temperature of the room, and with another heated over a spirit-lamp. The same changes will occur in both, but much more quickly in that which is heated. A precipitate falls when either solution is neutralized.

7. Concentrated Sulphuric Acid : The albumin dissolves, and more quickly if heated.

†8. Concentrated Nitric Acid : The albumin dissolves, forming a yellowish solution. When boiled it dissolves more quickly. When the solution is allowed to cool, and ammonia added to it, it becomes orange-coloured.

** 8. **Coagulation of Albumin.**—One of the most remarkable properties of albumin is its precipitation from neutral solutions as an insoluble coagulum by boiling.

In heating albuminous solutions care must be taken not to hold them too near the flame, and also to shake or stir them about, as otherwise the coagulum sticks to the tube and becomes burnt, and the test-tube cracks. Boil a watery solution of albumin in a test-tube; a coagulum separates.

Alkali appears to be set free during Coagulation.—Add a few drops of neutral solution of litmus to a solution of albumin and boil. The colour will become more decidedly blue.

Circumstances which influence Coagulation. Temperature at which Coagulation occurs.—Although solutions of albumin are generally boiled in order to produce coagulation, it takes place much below the boiling point. The temperature at which it occurs sometimes serves to separate albuminous bodies which could not otherwise be distinguished (*see* § 60). The method of ascertaining it is as follows: Put some aqueous solution of albumin in a test-tube; place it along with a thermometer in a beaker containing water, and apply heat very gradually till coagulation begins to take place and the solution grows milky from the formation of a precipitate. Then note the temperature of the water. If the liquid is heated over a naked flame, it cannot be so equally and gradually warmed throughout, nor the temperature at which coagulation occurs so accurately ascertained.

Effect of Acids and Alkalis on the Temperature of Coagulation.—The addition of very dilute acetic or phosphoric acid causes coagulation to take place at a lower temperature. The addition of a very little sodium carbonate prevents coagulation from taking place till the solution has been raised to a higher temperature than is necessary in neutral solutions. A large quantity will prevent it altogether.

Put some albumin solution into three test-tubes, acidulate one slightly with very dilute acetic or phosphoric acid, add to another a drop or two of a solution of sodium carbonate, and keep the third without any addition, for the purpose of comparison. Put a drop or two of litmus solution into each, so that they may be distinguished by their colour, or attach a small label to each. Place all three in a beaker, and warm them as in the previous experiment. As the temperature rises coagulation will occur, first in the acid, next in the neutral, and lastly in the alkaline solution.

Effect of neutral Alkaline Salts on the Temperature of Coagulation.—The addition of neutral alkaline salts, such as sodium chloride or sulphate, to a solution of albumin causes it to coagulate at a lower temperature than it would otherwise do. The salts produce this effect in neutral, in acid, and in alkaline solutions of albumin.

Repeat the previous experiment, dividing each solution into two parts and adding to one of them some saturated solution of sodium sulphate. In each case coagulation will take place at a lower temperature in the solution to which the salt has been added than in the corresponding one to which no addition has been made.

As the acetic acid alone lowers the temperature of coagulation and the addition of neutral salts does so still farther, the solution to which both have been added will coagulate first. By adding a large quantity of the salt and of acetic acid coagulation may be produced at a temperature between 20° C. and 30° C. (Hoppe-Seyler).

† *Coagulation is not due to heat alone, but to the presence of Water.*—Take some perfectly dry albumin, put it in a test-tube, cover the mouth of the tube and plunge its lower end into boiling water. Keep it there sufficiently long to be certain that the albumin has been heated to 100° C. Take it out, let it cool, and then add water to the albumin. It will be found soluble. Plunge the tube a second time into the boiling water, and the solution will be coagulated.

** 9. **Precipitation of Albuminous Bodies.**—Though the action of the following reagents may be conveniently tried with a solution of egg albumin, their power of precipitating albumin is not limited to that obtained from eggs, but extends equally to all other albuminous bodies.

Take a solution of albumin in water, put some of it into ten test-tubes, and add the following reagents. They all precipitate albumin.

†1. Concentrated nitric acid.

2. Concentrated hydrochloric acid.

3. Concentrated sulphuric acid.

†4. Acetic acid, or a little hydrochloric acid, and afterwards a solution of potassium ferrocyanide.

5. Acetic acid and a considerable quantity of a concentrated solution of sodium sulphate. [Other neutral salts of the alkalis or alkaline earths as well as gum arabic or dextrin have a similar action to sodium sulphate.]

6. Basic lead acetate.

7. Mercuric chloride.

8. Tannic acid.

9. Powdered potassium carbonate thrown into the solution till it is almost saturated.

10. Alcohol.

** 10. **Detection of Albumin.**—The three tests ordinarily used to detect the presence of albumin in a fluid are

1st. Its precipitation when boiled and acidulated with nitric acid.

2nd. Its precipitation by acetic acid and ferrocyanide of potassium.

3rd. Its precipitation when boiled with acetic acid and a strong solution of neutral salt.

The student should first try these tests with a solution known to contain albumin, so as to become familiar with them, and afterwards with a solution which may or may not contain it.

1. Put some of the fluid in a test-tube and heat it over a spirit-lamp or Bunsen's burner till it boils. Add a drop or two of nitric acid so as to give it a most distinctly acid reaction. If a precipitate is formed by boiling and is unchanged by the nitric acid, or if one forms after the addition of the acid, the fluid contains albumin.

The acid is added for two reasons. (*a*) To dissolve any substance which might be present in the solution, and being precipitated by boiling might simulate albuminous coagulation. Such substances are calcium phosphate which is present in human urine, and calcium carbonate in the urine of herbivora. As this test is very frequently used for detecting albumin in urine, these substances might very easily lead to error. Albumin which has been coagulated by heat is not soluble in nitric acid, and if the precipitate produced in the fluid by boiling disappears on the addition of acid, no albumin is present.

(*b*) To neutralize alkali which might hinder the albumin from being precipitated by boiling.

Take some solution of albumin in water, add a few drops of liquor potassæ and boil. No precipitate occurs. Add one drop of dilute nitric acid—any precipitate which forms disappears on shaking the tube. Add sufficient to make the fluid very distinctly acid, and a permanent coagulum will be produced. The quantity of acid added must therefore not be too small, or some albumin may remain in the solution. Sometimes, instead of using nitric acid, the fluid is kept boiling, and acetic acid added very gradually till the fluid is neutral. Unless very great care is taken to neutralize the fluid exactly, this method may fail, for if an excess of acetic acid be added it will retain the albumin solution. If neutralized exactly, the albumin will be precipitated, and may be separated from the fluid by filtration.

2. Acidulate the fluid strongly with acetic acid, and then add several drops of a solution of potassium ferrocyanide. If albumin be present, a white flocculent precipitate will occur.

3. Add acetic acid to the fluid till it is very distinctly acid,

mix it with its own volume of a strong solution of sodium sulphate, and heat to boiling. If albuminous bodies are present, a permanent precipitate will be formed.

This last method enables us not only to discover albumin when present, but to separate it from the solution, so that tests for other substances, such as sugar, with which the presence of albumin would have interfered, may then be applied to it.

11. Separation of Albuminous Bodies from other Substances in solution.—1. The usual way of separating albuminous bodies from solutions is by boiling, so as to coagulate the albumin. If the solutions are already acid, they are boiled without adding anything, but if not, a little dilute acetic acid is to be added before boiling, excess being carefully avoided.

2. If complete coagulation is not produced by boiling with acetic acid alone, an equal volume of concentrated solution of sodium sulphate may be added, and the liquid again boiled.

** **12. Tests for traces of Albumin in solution.**—1. *Caustic Potash and Copper Test.*—It is advisable, before trying this test, which is also used for the detection of sugar, to become acquainted with the reaction presented when a caustic alkali is added to a solution of cupric sulphate, and the mixture heated without any foreign substance being present in the solution. Put a little distilled water into a test-tube, with a drop or two of a dilute solution of cupric sulphate. Pour into it some liquor potassæ, and a light blue precipitate of hydrated cupric oxide will be thrown down. Boil the liquid, and the blue precipitate will be converted into a black powder, which is anhydrous cupric oxide. If it is gently warmed, instead of boiled, the powder will be dark brown.

The hydrated cupric oxide is not soluble in excess of ordinary liquor potassæ, but is slightly soluble in very concentrated solutions of potash, and imparts to them a light blue colour. The presence of certain organic substances renders hydrated cupric sulphate soluble in weaker alkaline solutions. Put some water and cupric sulphate solution in a test-tube; add a small crystal of tartaric acid, or a few drops of its solution, and then liquor potassæ. Either no precipitate will form, or it will redissolve, and, on shaking the tube, the liquid will become of a bright blue colour. Boil it: no precipitate will fall, and no change in the colour will take place.

Application of this Test to Albumin.—Put some solution of albumin in a test-tube; add a drop or two of cupric sulphate and liquor potassæ; an excess of liquor potassæ does not interfere with the reaction. Either no precipitate will fall, or it will be dissolved on shaking the tube, the liquid assuming a violet colour. Boil it. No precipitate falls, but the violet colour will become deeper.

** 2. *Xanthoprotein Reaction.*—Add to the fluid some concentrated nitric acid, and boil. Let the liquid cool, and then add a little ammonia. If albumin is present, an orange colour will be produced. This is one of the most delicate tests for albuminous substances.

** 3. *Millon's Reaction.*—Add to the fluid a little of Millon's reagent and heat. If albumin be present in considerable quantities, a white precipitate will fall and become red on heating; if only a trace be present, the fluid will become red. The red colour is produced even at ordinary temperatures, but it is increased by heating.

To prepare Millon's reagent take two beakers, one of which may be considerably larger than the other; place one on each pan of a pair of scales, and add shot or weights to the pan containing the lighter beaker till the other is counterbalanced. Pour into the smaller beaker a little mercury, and into the other the same weight of nitric acid (sp. gr. 1042). Dissolve the mercury in the nitric acid at first without, and afterwards with gentle warmth. Pour the solution into a graduated glass measure, and add to it twice its volume of water. Let it stand for some hours, and then decant the fluid from the crystalline deposit.

SECTION II.—ALTERATION OF ALBUMIN BY ALKALIS.

Egg albumin is converted into alkali-albuminate when it is dissolved in caustic potash or soda, or when they are added to its solutions. Alkali albuminate is the substance first described by Mulder under the name of protein. He considered it to be the essential part of all albuminous bodies, and regarded them as compounds of it.

Albuminous bodies are not converted immediately into alkali albuminate, but they undergo this change when allowed to stand with caustic alkalis, and it is greatly accelerated by the application of heat.

Alkali albuminate is not coagulated by heat. It is soluble in weak alkalis. It is precipitated when the alkaline solutions are neutralized by acids. It is soluble in very dilute acids, especially hydrochloric acid, and when the acid is added in excess to an alkaline solution, the precipitate which was thrown down by its neutralization is again dissolved very readily by the acid. When the acid solution is neutralized by an alkali the albumin is again precipitated.

If the alkali albuminate is precipitated by neutralization, and the precipitate immediately dissolved in acid, it is quickly converted into syntonin. If it is precipitated, and allowed to stand for some time, it will still be dissolved by dilute acids, but not so readily as immediately after precipitation, and it must be warmed with them to 60° C in order to convert it into syntonin.

If alkaline phosphates are present in the solution, alkali albuminate is not precipitated by neutralization. When just sufficient acid has been added to a solution of alkali albuminate to convert the basic phosphate into acid phosphate, the slightest excess of acid, or even CO_2 will produce a precipitate. In studying the action of alkalis on albumin, it is as well to begin with their action on solution of albumin, and afterwards to examine the solid alkali albuminate.

** 13. **Alkali Albuminate.**—Dissolve some albumin in water in a beaker; add to it a few drops of liquor potassæ, and put a little of the mixture into four test-tubes. *The Alkali Albuminate is not formed immediately.*—To the solution in the first tube add a drop of watery solution of litmus (*see* App. § 217). Then add very dilute acid till the blue colour of the litmus begins to change to red. No precipitate, or only a very slight one, will take place. Boil the neutral liquid; a precipitate is produced showing that much unchanged albumin is still present. If a precipitate falls, the presence of much unchanged albumin may still be demonstrated by filtering and boiling the filtrate, or adding tannin to it, when a precipitate will be produced. *It is quickly formed when heat is applied. It is not coagulated by boiling.*—Gently warm the fluid in the second test-tube till it boils; no precipitate forms. Let it cool, add a drop of litmus to it, and neutralize. Just when the blue begins to change to red the fluid will become turbid from the precipitation of the alkali albuminate. Let the precipitate settle, and filter the fluid. Boil the filtrate; no precipitate is formed, showing that the whole of the albumin has become insoluble in water, and has been precipitated by neutralization. *It is soluble in dilute acids.*—Warm and neutralize the solution in the third tube as in last experiment, then add an excess of hydrochloric acid to the neutralized solution, and the liquid will again become clear. On neutralizing the solution a second time the precipitate re-appears. *It is formed at ordinary temperatures, but more slowly.*—Let the solution in the fourth tube stand for some time, and then neutralize it. The precipitate will be greater than in that which was neutralized immediately after adding the potash. Filter and test the amount of albumin in the filtrate by adding tannin. It will vary, being greater or less, according to the shorter or longer time the solution has been allowed to stand.

* 14. **Preparation of Solid Alkali Albuminate**.—*a*. From eggs. Put the white of one or two eggs in a beaker, cut it up with scissors and shake it vigorously with air in a flask until the membranes separate and come to the top with the foam. Filter it through a piece of linen. Add strong solution of caustic potash to it drop by drop, until the whole mass becomes transformed into a stiff jelly. Cut it into pieces about the size of a horse-bean and throw them into a large quantity of distilled water. Stir them round and round a few times and then pour off the water, keeping back the pieces by a piece of gauze stretched across the mouth of the beaker. Wash the albuminate with fresh water several times in order to remove the free alkali until the pieces begin to turn white at the edges and exhibit only a faint though distinct alkaline reaction. As the albuminate is soluble in water containing alkali, a good deal of it is lost in the process. When deprived of its alkali by prolonged washing or by soaking in dilute acids it forms *pseudo-fibrin.* Like fibrin this substance is elastic, and it swells but does not dissolve in dilute hydrochloric acid. Unlike fibrin it contains no ash, and when put into hydrogen peroxide does not readily decompose it; so that few bubbles of gas appear.

b. From milk. Alkali albuminate may be prepared from milk by shaking with caustic potash and ether, removing the ether, precipitating the albuminate by acetic acid and washing the coagulum with water, alcohol, and ether.

15. **Properties.**—Boil some pieces of albuminate in water; it still contains alkali, and is soluble in boiling water, forming a feebly alkaline solution. Let it cool, divide it into several portions and apply the following tests :—

1. Pass CO_2 through the solution and a precipitate will fall. No precipitate is produced if the solution is *strongly* alkaline.
2. Add alcohol to the solution; no precipitate is produced.
3. Add magnesium sulphate in substance till the solution is saturated. The albuminate will be precipitated. Calcium chloride will have the same effect.

It is precipitated by metallic salts like other albuminous solutions. It is precipitated by neutralization, and behaves to alkaline phosphates like the solution of alkali albuminate prepared by heating solutions of albumin with potash.

* *It is not precipitated by Neutralization in presence of Alkaline Phosphates.*—A very small quantity of acid is sufficient to give a distinctly acid reaction to a pure solution of alkali albuminate, but if sodium or potassium phosphate is present a considerable amount of dilute acid may be added to the liquid after the point of neutralization has been reached

without giving it a very distinctly acid reaction; for the acetic acid and neutral phosphate react on each other, forming sodium or potassium acetate and acid phosphate. Whenever the solution becomes distinctly acid, the albumin is precipitated, whether sodium phosphate be present or not. If sufficient acid has been added to convert all but a trace of the sodium phosphate present into acid phosphate, the further addition of CO_2 will cause a precipitate. Heating the solution will also cause a precipitate, for it converts the acid phosphate into neutral phosphate, and by thus liberating free acid acts just as the addition of more acid would do.

Put some solution of alkali albuminate into two test-tubes; add solution of sodium phosphate to one of them, and colour them both equally with solution of litmus.

Neutralize them both with very dilute acetic acid. Very little acid will neutralize the pure solution of albuminate, and the slightest excess will at once turn the litmus red. A greater quantity may be added to the other without turning it red, and till it turns red no precipitate will fall.*

16. Alkali Albuminate should contain no Sulphur.—The sulphur which is contained in albumin is said to be removed by the alkali used in converting it into alkali albuminate, and it therefore differs from casein and syntonin, in both of which sulphur is present. The presence of sulphur is thus tested: Put a piece of alkali albuminate into liquor potassæ, add a drop of solution of lead acetate and boil. The solution should not become brown, as it would do from the formation of lead sulphide, if sulphur were present in the alkali albuminate. The sulphur is, however, by no means always removed during the preparation, and it is very probable that a brown colour will be got.

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* It is usually stated that alkali albuminate is precipitated by neutralization. In the text I have made use of this expression, which is perhaps a convenient one, since the quantity of acid necessary to produce precipitation being extremely small and the precipitate soluble in excess, the direction to neutralize rather than to acidulate is more likely to lead to the desired result. I believe the student will readily convince himself that alkali albuminate is not precipitated from its solutions by exact neutralization, and is only thrown down when a slight excess of acid is present. I am inclined to think that the sodium phosphate acts simply by preventing the inadvertent addition of a slight excess of acid, which is extremely liable to occur in solutions of alkali albuminate, and that syntonin is precipitated by neutralization in presence of sodium phosphate, while alkali albuminate is not, because the point of slight acidity at which the albumin is precipitated is reached before that of neutralization in the former case, so that, before neutralization is effected, the albumin is thrown down; while in the latter, the solution does not become acid, and the albumin is therefore not precipitated, till after neutralization. On this subject compare Rollett, Wien. Sitz. Ber. XXXIX. p. 547, and Moleschott's Untersuch, VII. p. 230, also Soxhlet, Journ. f. pract. Chemie, N. F. 1872, VI. p. 1.

SECTION III.—ALTERATION OF ALBUMIN BY ACIDS. ACID ALBUMIN OR SYNTONIN.

When a solution of albumin is treated with very dilute acids, or when solid albumin is dissolved in concentrated acids, it is converted into acid albumin, which is identical with syntonin, or, at any rate, appears to be so. Myosin, vitellin, and fibrin are quickly dissolved by dilute acids, and converted into syntonin. It is soluble in very dilute acids, but is insoluble in water, and it is, therefore, precipitated by neutralization. It is redissolved by excess of alkali, as it is soluble in alkalis and alkaline carbonates. Unlike alkali albuminate, its precipitation is not prevented by the presence of alkaline phosphates. It is not precipitated from decidedly acid solutions by boiling, but when the solutions are nearly neutralized, and only very faintly acid, boiling precipitates it.

** 17. **Preparation of a Solution of Acid Albumin or Syntonin.**—Put some solution of albumin in water into a beaker and mix it with its own bulk of dilute hydrochloric acid (four cubic centimeters of strong commercial acid in one litre of water). Pour some of the mixture into several test-tubes. *The dilute acid does not convert the albumin immediately into syntonin.* Add to the fluid in the first test-tube a drop of litmus solution, and then neutralize it exactly with dilute liquor potassæ. Little or no precipitate will fall. If any should be produced, filter, and boil the filtrate or add tannin to it. A copious precipitate will appear, showing that there is much albumin in the solution. *The prolonged action of the acid converts it into syntonin, which is precipitated by neutralization.* Let one tube stand for some hours, and then examine it, or prepare it some hours before, and examine it at the same time as the rest. Put a drop of litmus into it, divide the liquid into two parts and then neutralize one part exactly. The whole of the albumin will be precipitated from the liquid. To show this, filter and boil the filtrate no precipitate will be produced. *Acid albumin is not precipitated from acid solutions by boiling.*—Boil the other part of the liquid. The albumin in it has been already shown to be converted into syntonin. No coagulation will occur. *The formation of acid albumin is accelerated by heat.* Warm a test-tube containing albumin solution mixed with acid gently to boiling. Add a drop of litmus solution, and neutralize. The albumin will be completely precipitated, and the solution, when filtered, will give no precipitate on boiling.

† *Syntonin is precipitated from its solutions by neutraliza-*

tion, even though alkaline phosphates be present.—Repeat the last experiment, adding a little sodium phosphate before neutralizing. The syntonin will be precipitated as before.

** 18. **Behaviour of Syntonin with Acids.**—Syntonin is soluble in concentrated mineral acids; it is insoluble in them when they are moderately dilute, and it is soluble in them when very dilute.

Heat a watery solution of albumin gently to boiling, with its own bulk of very dilute hydrochloric or nitric acid (four parts of commercial acid in 1,000 of water). No coagulum will be produced. Add a small quantity of strong acid and a precipitate will form which will dissolve in a large quantity of the acid, especially when heated.

Put a little serum albumin into three test-tubes, and add to one concentrated nitric acid, to another hydrochloric, and to a third sulphuric acid. Dissolve the albumin in the acid by heating.

Dilute the solutions with twice their volume of water, and a precipitate will fall. Let it settle, and pour off the supernatant liquid or filter it; throw the precipitate, still moist with acid, into water, and it will dissolve. This is not a solution in water, but in dilute acid, for a considerable quantity of acid still remains in the precipitate. Egg albumin differs from serum albumin in its behaviour with acids, and this, and its coagulability by ether, form the chief distinctions between them.

Repeat the last experiment with egg albumin. It will not dissolve so readily in nitric or hydrochloric acid, and when precipitated by dilution will dissolve slowly and imperfectly in more water instead of doing so readily, like serum albumin. The precipitate from hydrochloric acid will be brittle and fibrous if the solution has been recently made, but if the solution is boiled until it begins to become violet, or allowed to stand for some days, the precipitate will be flocculent and soluble, like that of serum albumin.

* 19. **Preparation of Syntonin.**—(*a.*) From serum or egg albumin. Neutralize the solution in dilute acid, obtained in last experiment, with dilute liquor potassæ; a gelatinous flocculent precipitate of pure syntonin will fall.

(*b.*) From fibrin. Dissolve it in concentrated hydrochloric acid; filter the solution if necessary, and then proceed as with serum albumin.

(*c.*) From muscle. Mince some muscle, wash it with water, add to it a considerable quantity of dilute hydrochloric acid (four cubic centimeters of strong acid to one litre of water), and let it stand for several hours, stirring it frequently. Filter it through a plaited filter. Dilute the filtrate with water, neutralize it with a solution of sodium carbonate, and wash the precipitate with water.

20. Characters.—When freshly precipitated, syntonin forms a sticky jelly, but it is not tenacious.

Solubility.— It is insoluble in water, and in dilute NaCl solution. It is readily soluble in lime-water, in dilute hydrochloric acid and weak alkaline solutions. It is not soluble in a solution of six parts of potassium nitrate in 100 of water.

Its solutions behave like those made by heating albuminous solutions with dilute acids.

21. Tests.—Dissolve some syntonin in lime-water and boil it. Coagulation will occur.

Add magnesium sulphate or calcium chloride to a cold alkaline solution of syntonin. Unlike alkali albuminate, it will not be precipitated. Boil the solution, and precipitation will occur.

Boil an alkaline solution of syntonin, and then add magnesium sulphate or calcium chloride, and a precipitate will fall at once. This would seem to be due to the syntonin being converted into alkali albuminate by boiling.

22. Syntonin contains Sulphur.—Dissolve some syntonin in liquor potassæ, add a drop of a solution of lead acetate to it, and boil. It will become brown from the formation of lead sulphide.

23. Distinction between Alkali Albumin and Syntonin.—If a solution of alkali albuminate contains an alkaline phosphate, the alkali albumin is not precipitated when the solution is neutralized, but syntonin is precipitated from its solutions by neutralization whether an alkaline phosphate be present or not.

SYNOPSIS OF THE CHIEF ALBUMINOUS BODIES. (Hoppe-Seyler.)

24. I. Albumins.—Albuminous bodies which are soluble in water and are not precipitated by very dilute acids, alkaline carbonates, NaCl, or platino-hydrocyanic acid. Their solutions are coagulated by boiling.

1. *Serum Albumin.* Not coagulated by shaking with ether. Readily soluble in concentrated hydrochloric acid; water added to this solution causes a precipitate which is readily dissolved by more water.

2. *Egg Albumin.* Precipitated by ether. Less readily soluble in concentrated hydrochloric acid: water added to this solution causes a precipitate which dissolves with difficulty in a large quantity of water.

25. II. Globulins.—Albuminous substances, insoluble in water, soluble in dilute NaCl solution. The solution is coagulated by heat. Very dilute hydrochloric acid dissolves them and converts them into syntonin.

1. *Vitellin.* Not precipitated by the addition of NaCl in substance to the solution until it is saturated.

2. *Myosin.*—Precipitated from its solution in dilute NaCl solution by the addition of NaCl in substance.

3. *Fibrinogenic Substance* and—

4. *Fibrinoplastic Substance* (*Paraglobulin*) agree with myosin in their reactions, but together in neutral solutions they form fibrin.

26. **III. Fibrins.**—Insoluble in water or in NaCl solution. In dilute acids they swell, and also in solutions of soda, though to a less extent. The swollen substance is coagulated by heat.

27. **IV. Albuminates.**—Insoluble in water or in NaCl solution. Easily soluble in very dilute hydrochloric acid and in alkaline carbonates. The solutions are not altered by boiling. They are not precipitated from their solutions by neutralization if alkaline phosphates are present.

1. *Casein* yields potassium sulphide when allowed to stand with liquor potassæ, and still more quickly when heated with it.

2. *Alkali albuminates* (*Proteins*) do not yield potassium sulphide with liquor potassæ.

28. **V. Acid Albumins, or Syntonin.**—Insoluble in water or in NaCl solution. Easily soluble in dilute hydrochloric acid, Precipitated from solution by neutralization, even in presence of alkaline phosphates.

29. **VI. Amyloid.**—Insoluble in water, dilute hydrochloric acid, and sodium carbonate; in solutions of NaCl it does not perceptibly swell. It is coloured reddish-brown or violet by iodine. It is not digested by gastric juice at the temperature of the blood.

30. **VII. Coagulated Albuminous Bodies.** — Insoluble in water, very dilute hydrochloric acid and sodium carbonate; in NaCl solutions they do not swell up perceptibly. They are coloured yellow by iodine. They are readily converted into peptones by gastric juice at the temperature of the blood.

31. **VIII. Peptones.** — Soluble in water, not precipitated from the solution by acids, alkalis, or heat.

33. **Decomposition of Albumin.**—The decomposition of albumin by various agencies is of great interest, as it is only by a study of the way in which it splits up that a knowledge of its constitution can be obtained.

When treated with powerful oxidizing agents albuminous

32. ALBUMINOUS BODIES IN SOLUTION.

Coagulable by heat	Not precipitated by CO_2 Not precipitated by very dilute hydrochloric acid Not precipitated when the solutions are saturated with NaCl	Albumins.	Precipitated by shaking with ether	Egg Albumin.
			Not precipitated by ether	Serum Albumin.
	Precipitated by CO_2 The precipitate produced by CO_2 is soluble in very dilute solutions of NaCl	Globulins.	Not precipitated by saturating the solution with NaCl in substance	Vitellin.
			Precipitated by saturating the solution with NaCl in substance	Myosin. Fibrinogenic substance. Fibrinoplastic substance.
Not coagulated by heat	Precipitated by neutralizing with acetic acid. Precipitation prevented by the presence of alkaline phosphates	Albuminates.		Casein.
				Alkali albuminates.
	Precipitated by neutralization. Precipitation not prevented by the presence of alkaline phosphates	Acid Albumins (Syntonin).		Acid albumin or syntonin.
	Not precipitated by CO_2: not precipitated by neutralization, but precipitated by alcohol	Peptones.		Peptones.

bodies yield formic, acetic, propionic, butyric, valerianic, caproic, and benzoic acids and the corresponding aldehydes, ammonia, and volatile organic bases.

Such substances are, however, too far removed from albumin; it is not from these final products of its decomposition that much information is to be got, but rather from those bodies of a tolerably complex nature into which it first splits up when treated with less active decomposing agents. These may afterwards undergo further decomposition, and yield substances of a simple constitution.

The most important decomposition is that which albuminous bodies undergo when boiled with water or with acids, or when subjected to the action of one of the pancreatic ferments. Under such circumstances peptones are first formed, and afterwards split up, yielding leucine and tyrosine.

34. **Peptones.**—These are distinguished from other albuminous bodies by not being precipitated by boiling, by alkalis or acids, nor by acetic acid and potassium ferrocyanide. They are precipitated by alcohol. Unlike albumin, they diffuse easily through vegetable parchment. With caustic potash, and a trace of cupric sulphate, they give a precipitate, which dissolves on shaking, and forms a solution of a red colour, becoming violet on the addition of more copper sulphate.

Bodies which closely resemble the peptones formed during digestion may be prepared by boiling albuminous bodies, such as fibrin, for a long time with water, especially under pressure, in a Papin's digester, in a sealed glass tube, or in a soda-water bottle. By boiling with dilute sulphuric acid or concentrated hydrochloric acid, they are produced in a shorter time. The production of peptones by the digestive ferments will be considered afterwards.

35. **Leucine.**—*Preparation.*—It may be obtained by boiling fibrin with dilute acid for a long time, or by digesting it with pancreas, but it is more usually got from horn chips. Boil two parts of horn shavings with five parts of sulphuric acid, previously diluted with thirteen parts of water, for twenty-four hours, loss of water by evaporation being prevented by the arrangement shown in fig. 329. Saturate the fluid while hot, with chalk, filter, evaporate the filtrate to half its bulk, add oxalic acid to precipitate the lime, filter and evaporate till a scum forms on the surface, and then set it aside to crystallize. A considerable amount of tyrosine will crystallize out first. Pour off the liquor, let it stand till crystals of leucine form. Purify them by boiling with water and lead hydrate, filter, remove the lead by sulphuretted hydrogen, filter, evaporate the filtrate in a

water bath to dryness; dissolve the residue in hot weak alcohol, and let it cool and evaporate till crystallization takes place.

Leucine can be formed synthetically, and if wanted pure, this is the best way of obtaining it.

For this purpose a mixture of valeral-ammonia, hydrocyanic and hydrochloric acids are boiled together in a retort till the oily ammonium compound has disappeared. The liquid is then evaporated to dryness, the residue is boiled with water and lead hydrate, and the product purified as already directed.

Characters.—Leucine forms extremely slender white, glistening plates. Allow a drop of a solution in water or alcohol to evaporate on an object-glass and examine it under the microscope. It will form round balls, which are either hyaline, and strongly resemble fat globules, or exhibit radiating lines. Or it may appear as very thin plates grouped in a radiating fashion. They differ from urates presenting a similar form in not being strongly refractive.

Solubility.—1. Water: Pure leucine dissolves slowly, and is soluble in about twenty-seven parts of cold water. It dissolves more easily in hot water. When impure it is more easily soluble.

2. Alcohol: Pure leucine dissolves in 1040 parts of cold, and in 800 of hot alcohol. If impure, it is much more soluble.

3. In Liquor potassæ, 4, ammonia, and 5, dilute acids, it is readily soluble.

6. Concentrated hydrochloric or sulphuric acids. It is dissolved without decomposition. Neutralize them, and it is precipitated.

† *Effect of Heat.*—At 170° C it sublimes unchanged; a higher temperature decomposes it.

Put a little leucine into a dry test-tube and heat it gently. It will rise in white clouds and be deposited on the cool part of the tube. Heat the deposit strongly, and a strong smell of amylamine will be perceived.

Decomposition.—When decomposed by heat it yields CO_2 NH_3, and amylamine.

To show this put a portion of leucine into a hard glass bulb, and connect this by means of india-rubber tubing with a glass tube long enough to reach to the bottom of a test-tube. Prepare two other similar pieces of glass tubing and three test-tubes, the first of which should be about half filled with caustic baryta solution, the second with Nessler's reagent, and the third with water. Heat the bulb containing the leucine, applying the heat first to the upper part of the bulb and gradually moving it downwards, so that as the leucine sublimes its vapour may be strongly heated and decomposed. Pass the fumes into the baryta solution, then disconnect the glass tubing, and after attaching a clean piece, pass them into Nessler's reagent and then into water. The baryta will be precipitated as white carbonate, the Nessler's reagent will become brown, showing the presence of ammonia, and the water in the third test-tube will acquire the peculiar smell of amylamine and an alkaline reaction. Add to the barium solution a little nitric acid. It will become clear and evolve gas, showing that the precipitate was barium carbonate. A minute quantity only of NH_3 is disengaged when leucine is heated alone, and the coloration of Nessler's reagent is therefore very slight. If a little lime and caustic soda or potash are heated with the leucine much more NH_3 is given off.

36. **Preparation of Nessler's Reagent.**—Dissolve 4 grammes of potassium iodide in 250 cub. cent. of distilled water. Set aside a few cub. cent., and add a cold saturated solution of mercuric chloride to the remainder, till the precipitate of mercuric iodide is no longer dissolved on stirring. Add that part of the potassium iodide solution which was set aside, to the rest, so as to dissolve the remaining precipitate, and then add mercuric chloride again very gradually, till a slight permanent precipitate is produced. If a few cub. cent. of the potassium iodide solution were not set aside, great caution would be required in adding the mercuric chloride so as to avoid excess. Dissolve 150 grammes of potassium hydrate in 150 cub. cent. of distilled water, allow the solution to cool, and add it gradually to the potassium iodide solution. Pour the mixture into a measuring-glass or flask, and add distilled water to make up a litre. Pour it into a large well-stoppered bottle, taking care that there is no ammonia near it at the time. It will deposit a brown precipitate, and become quite clear and of a pale greenish-yellow colour. It is then ready for use; a little of it should be poured into a smaller bottle when wanted.

37. **Detection of Leucine in Tissues.**—In order to detect the presence of leucine, cut up the organ (the pancreas of a sheep or ox, for example) into small pieces with a large knife or sausage-making machine. Mix it with water and let it stand for a little while, stirring it frequently; filter it through a piece of cloth, and press out the water first with the hand, and then with a screw-press. Extract it with water a second time in the same way. Mix the watery extracts together, acidify slightly with acetic acid, and boil, to coagulate the albumin. Filter: add a solution of lead acetate to the filtrate. Filter: pass sulphuretted hydrogen through the filtrate to remove the excess of lead. Filter: evaporate the filtrate to dryness. Extract the residue with boiling alcohol. Filter: evaporate the filtrate to a syrup,

and set it aside for several days to crystallize. If leucine is present, it will crystallize in a day or two in balls or knots, or, possibly, in shining plates, but will not form good crystals. It is not pure, but is mixed with a number of other substances. In order to free it from these, the following method is recommended by Hoppe-Seyler. Dissolve it in ammonia, add lead acetate till no further precipitate is produced. Filter: wash the precipitate with a little water. Suspend it in water, and pass sulphuretted hydrogen through it. Filter, and evaporate the filtrate in the water bath.

38. **Tests for Leucine.**—The formation of round lumps or plates is not sufficient to prove that a substance is leucine, and other tests must be applied to them. Before doing so, they should be purified by drying them between two folds of blotting-paper, dissolving them in boiling alcohol, and letting them crystallize out again. The following tests may be applied:—

1. Put a portion into a dry test-tube and heat it over a Bunsen's burner or spirit-lamp. If it consists of leucine it will emit the smell of amylamine.

2. Scherer's Test: Put a small portion of the supposed leucine with a drop of nitric acid on a piece of platinum foil, and evaporate it gently. If it is pure leucine, a colourless, almost invisible, residue will remain on the foil. Add a few drops of liquor potassæ to it, and heat. It will become yellow or brownish, and then form an oily drop, which runs about upon the foil without adhering to it.

39. **Tyrosine.**—*Preparation.*—Boil horn shavings with dilute sulphuric acid, crystallize out the tyrosine, as directed in the preparation of leucine, wash the crystals with cold water, dissolve them in ammonia, and allow the solution to evaporate, until the tyrosine crystallizes.

It forms fine colourless microscopic needles, with a silky lustre, and without taste or smell.

Or digest fibrin with pancreas, *see* § 171.

Characters.—Let a drop of a solution of tyrosine in hot water evaporate on an object-glass, and examine it under the micro scope. Long needle-like crystals will be seen which are often united in single tufts, or in radiating groups of tufts.

Solubility.—1. Cold water dissolves it with difficulty. 2. Boiling water dissolves it easily. Almost all the tyrosine crystallizes out on cooling. It is insoluble in, 3. Absolute alcohol, 4. Ether. It is easily soluble in, 5. Ammonia, 6. Liquor potassæ, 7. Concentrated solution of potassium or sodium carbonate, 8. Alcoholic solution of caustic potash, 9. Concentrated hydrochloric or sulphuric acid, and, 10. Dilute mineral acid.

11. Acetic acid dissolves it with difficulty. 12. Nitric acid dissolves it. Let the solution stand a while. A yellow crystalline powder of nitro-tyrosine will separate. Pour off the liquid and add liquor potassæ to the powder. It will dissolve and form a red solution.

40. **Detection of Tyrosine.**—Treat the organ exactly as described in the process for the detection of leucine. The dried residue, after it has been extracted with boiling alcohol to remove the leucine, consists of tyrosine. Dissolve it in boiling water or ammonia, and let it crystallize out.

41. **Tests for Tyrosine.**—It is distinguished by its microscopic appearance, and by the following reactions.

1. Hoffmann's Test.—Put a little of the solution supposed to contain tyrosine in a test-tube; add some water, and a few drops of mercuric nitrate solution. Boil it for a little while. If tyrosine is present, the liquid will become rose-coloured, and will afterwards deposit a red precipitate.

2. Piria's Test.—Pour a few drops of concentrated sulphuric acid on two or three pieces of tyrosine the size of a pin's head in a watch-glass. Gently warm it for a little. Let the solution cool. Mix it with a little water, and add chalk or barium carbonate till all effervescence has ceased. Filter. Evaporate, if necessary, to a small bulk at a gentle heat, and add a few drops of a neutral solution of ferric chloride. The fluid will become of a beautiful violet.

3. Scherer's Test.—Put a little of the supposed tyrosine, with a drop or two of nitric acid, on a piece of platinum foil, and evaporate gently. If it is really tyrosine, it will quickly become of a bright yellow colour, and will leave a deep yellow shining residue. Add a few drops of liquor potassæ to it, and it will form a yellowish-red solution. Evaporate, and it will leave a brown residue.

CHAPTER XXXVI.

Chemistry of the Tissues.

42. **Epithelial Tissues.**—The epithelial tissues—nails, hair, epidermis, and epithelium, as well as horns and feathers—contain a small quantity of fat, and a substance which constitutes the chief part of their bulk, and to which their form is due. To this substance the name of *keratin* has been given. It is prepared by removing the fat, &c., from any of the epidermal tissues by boiling with ether, alcohol, water, and dilute acid. As the elementary analyses of it do not agree, it is quite possible that it is a mixture of several substances, but this is not yet certainly made out. It is nearly allied to albumin, as is shown by its yielding the same products, leucine and tyrosine, when decomposed by boiling with dilute sulphuric acid (*see* § 35). It contains sulphur, which seems to be in a very loose state of combination. Hair, as is well known, becomes blackened by lead sulphide when a leaden comb is used. To show the presence of sulphur, put a few parings of nails into a test-tube; add a little liquor potassæ, and boil. Add a little hydrochloric or sulphuric acid to the solution thus obtained. Hydrogen sulphide will be given off, and may be recognized by the smell.

43. **Connective Tissue.**—In the group of tissues so designated, there are several which do not seem very like one another. Such are mucous tissue, reticular and ordinary connective tissue, adipose tissue, cartilage, bone, and dentine. Their close relation to one another is shown by their being linked together by intermediate forms, by one tissue sometimes passing into another so that the boundary between them cannot be defined, and by one occasionally replacing another. They all contain substances which are either derived from albumin or are nearly connected with it, and have received the name of albuminoids.

44. *Albuminoids.* — These are nitrogenous, and resemble albuminous bodies in composition, but differ from them in their behaviour with acetic acid, potassium ferrocyanide, nitric and hydrochloric acids. They are mucin, gelatin, and chondrin.

** 45. **Mucin.**—This is found in fœtal connective tissue, and although not present in the fasciculi is an important constituent of tendon tissue. It occurs also in all mucous secretions, and gives them a tenacious character. It is distinguished by its solutions not being coagulated or rendered turbid by boiling; by giving with acetic acid a precipitate which shrinks together in pure acid, instead of swelling and dissolving as albuminous bodies do. The addition of potassium ferrocyanide to the acetic acid prevents it from precipitating mucin, so that no turbidity is produced unless albuminous substances are also present. It gives no precipitate with mercuric chloride; when heated with liquor potassæ and cupric sulphate, the solution remains of a clear blue.

Preparation. (*a*) *From Salivary Glands.*—Wash the salivary glands of an ox or sheep well. Cut them up into small pieces. Wash away any remaining blood with a little water. Mix the glandular substance well up with a considerable quantity of water, and filter through linen. Add acetic acid gradually to the filtrate, till a precipitate partly fibrous and partly flocculent is obtained. Filter through linen. Wash the precipitate with water, and then with alcohol and ether, to remove the fat.

(*b*) *From Tendons.*—Free the sinews of the legs of an ox or sheep from muscle. Wash them well, and cut them up in small pieces. Extract them with water. Put them into a large quantity of lime or baryta water, and let them stand in a closed vessel for several days. Filter. Add acetic acid in excess to the filtrate to precipitate the mucin. Wash the white flocculent precipitate with dilute acetic acid and then with dilute alcohol.

(*c*) *From Ox Gall.* (*See* § 134.)

Solubility. 1. Water:—It does not dissolve, but swells very much; when the mixture is filtered, part of the mucin often passes through, forming a turbid filtrate. The mixture with water is not tenacious, and no foam is produced on shaking it. 2. NaCl solution.—Add a little solid NaCl to a mixture of mucin and water. It will become clearer. Put a glass rod into the liquid. It will now be found to be tenacious, and on withdrawing the rod a long thread will follow it from the fluid. Shake it, and foam will form. Add a large quantity of water to the solution or mixture (for it is not certain which it is), and the mucin will be precipitated. 3. Very dilute hydrochloric acid of less than 1 per cent., or other mineral acid, does not dissolve mucin. 4. Dilute hydrochloric acid of 5 per cent. partly dissolves it. Shake the solution and it foams. Add a little NaCl to it, and the mucin will dissolve much more readily. 5. Concentrated hydrochloric, or other mineral acid, dissolves it completely. 6. Liquor potassæ dissolves it; add a little to some mucin, but not enough to dissolve the whole of it. Filter. The filtrate is not tenacious, and is *neutral.* 7. Baryta and lime-water dissolve mucin, and when used in small quantity give, like liquor potassæ, a neutral filtrate.

Precipitation of Mucin. † 1. Boil the neutral or slightly alkaline solution. It will not be altered.

† 2. Add acetic acid. A precipitate will fall. Let it settle. Pour off the liquid and pour on glacial acetic acid. Generally it will not dissolve.

† 3. Add acetic acid with solution of potassium ferrocyanide. If the mucin is pure no turbidity will appear at first, but will do so after the solution has stood for some time.

4. Add mercuric chloride. No precipitate.

5. Add basic lead acetate. A copious precipitate will form.

† *Reaction with Cupric Oxide.*—Add liquor potassæ and a little cupric sulphate to a solution of mucin. The cupric hydrate will be dissolved. Boil. The liquid will still remain of a clear blue colour. This distinguishes mucin from albumin, pepsine, and gelatin, which give a violet or red colour.

**** 46. Ordinary Connective Tissue.—Tendons.—Gelatigenous Substance, or Collagen.** — This substance forms the organic basis of bones and teeth, and the principal or fibrous part of connective tissue, tendons, ligaments, and fasciæ.

Preparation. (*a*) *From Bones.*—Soak some bones in hydrochloric acid diluted with 8 or 9 times its bulk of water, changing the acid several times. This will remove the inorganic salts which are deposited in the bone and impart hardness to it; so that when they are entirely removed, the bone will retain its original shape, but be quite soft and pliable. The time during which the bones must be soaked in order to remove the whole of the salts they contain, varies with their size; but if the bones be cut into small pieces, or thin bones such as ribs are used, a day or two is sufficient. Wash them well with water to remove the acid and dry them over the water bath.

(*b*) *From Tendons.*—After removing the mucin from tendons by means of lime or baryta water (*see* § 45), wash the swollen pieces first with water, and then with a little acetic acid much diluted, so that they contract and do not again swell. Then soak and wash them for a while in water, changing it several times.

Characters. —When fresh, it is soft, but it shrinks and becomes hard when it is dried or alcohol is added to it.

Solubility. 1. In cold water, it will not dissolve. 2. Boil the water. It will dissolve and be converted into gelatin. On cooling, it will form a jelly. 3. In cold dilute acetic or other acid, it will swell up. 4. In boiling dilute acids, it is dissolved and converted into gelatin still more readily than by water. 5. In hot liquor potassæ, it dissolves tolerably easily.

47. **Gelatin.**—*Preparation.*—Boil collagen obtained from bones or sinews in the manner already described. Filter the solution hot. Divide the filtrate into three parts. Allow one of them to cool; it will form a jelly. Evaporate another to dryness on the water bath. Use the third for testing various precipitants.

Solubility.—1. Cold water. Dried gelatin will swell, but will not dissolve. 2. Boil the water, it will dissolve. 3. Cold dilute acids, and 4. Cold dilute alkalis, will dissolve it readily.

Precipitation.—It is precipitated by 1, tannic acid; 2, mercuric chloride. Unlike albumin, pure gelatin is not precipitated by, 1, acetic acid and ferrocyanide of potassium; 2, many metallic salts, as lead acetate, cupric or ferric sulphate. It is not precipitated by acids or alkalis.

Alteration by Boiling.—Boil a solution of gelatin for some time with an acid or alkali and let it cool. It will remain fluid and will not form a jelly. Test its reactions. They will be found the same as before. The same effect is produced by prolonged boiling with water alone.

48. **Elastic Tissue.—Elastin.**—The elastic fibres which occur in the connective tissue in various parts of the body, and are especially abundant in the middle coats of the aorta and large arteries, and in the *ligamentum nuchæ*, and *ligamenta subflava*, are supposed to consist of elastin.

Preparation.—Remove the adhering cellular tissue from the fresh *ligamentum nuchæ* of an ox. Cut it into small pieces, and boil it with alcohol and ether to remove the fat. Boil it for 24 hours with water, to dissolve the collagen, renewing the water as it evaporates, or preventing evaporation (*see* § 207). Boil the residue with concentrated acetic acid for a considerable time; remove the acetic acid by boiling with water, and then boil with moderately dilute liquor sodæ or potassæ till it begins to swell. Remove the alkali by boiling with dilute acetic acid, then with water. Put the residue into cold hydrochloric acid; let it remain for 24 hours, and then wash it with water till the washings have no longer an acid reaction and leave no residue on evaporation.

Characters.—The elastin which remains after the treatment just described is yellowish and elastic while moist, but when dry becomes hard and brittle.

Solubility.—1. Put a piece of dry elastin in water. It will swell up but will not dissolve. 2. Boil the water. Unlike the collagen of connective tissue, it will not dissolve. It does not form gelatin, and the water will not gelatinize on cooling. 3. It does not dissolve in alcohol, ether, or acetic acid, though it swells in the latter. 4. Boil it with a strong solution of caustic potash; it will dissolve.

Precipitation.—Neutralize the solution in potash with hydrochloric or other acid. No precipitate will fall. Add tannin to the neutral solution. A precipitate will be produced. No other acids cause a precipitate.

Reactions.—1. Xanthoprotein reaction. Put a piece of elastin in concentrated nitric acid, and let it stay some time. It will swell up, then become yellow, and lastly form a mucilaginous solution. Add ammonia, and it will become a deep orange-red. 2. Millon's reaction. Test a piece of elastin with Millon's reagent. It will become slightly red.

Decomposition.—On boiling elastin with concentrated sulphuric acid it is decomposed and yields leucine, but no tyrosine.

** 49. **Cartilage.—Chondrogen.**—The intercellular substance of hyaline cartilage, and that which lies between the fibres of fibrocartilage consists mainly of chondrogen, so named because it is dissolved by boiling in water and forms chondrin.

Solubility.—Take a piece of costal cartilage of a sheep or ox and test its solubility in the following reagents:—1. Cold water. It is insoluble. When allowed to dry before it is put in water, it swells up slightly. 2. In boiling water, it dissolves. On cooling it forms a jelly. 3. In acetic acid it is insoluble. When dry, it swells very little in acetic acid.

50. **Chondrin.**—*Preparation.*—Boil the costal cartilages or trachea of a sheep or ox in water till the perichondrium strips easily off. Remove the perichondrium. Cut up the cartilages into very small pieces, and boil them with water for several hours. If a Papin's digester is at hand, boil them in it under a pressure of 2-3 atmospheres. Filter while hot. The filtrate will be strongly opalescent. Put part of it into a beaker and allow it to cool. It will form a jelly. To the remainder of the filtrate add acetic acid, and the chondrin will be precipitated.

Solubility.—Test the solubility of chondrin, using either that precipitated by acetic acid, or the jelly which formed on cooling, in the following reagents:—1. In cold water it is insoluble. Heat it, and it is dissolved. It is soluble in 2. Solutions of alkaline salts, as sodium sulphate, and is easily soluble in 3. Dilute mineral acids, 4. Liquor potassæ, and 5. Liquor ammoniæ. It is insoluble in 6. Alcohol, and 7. Ether.

Precipitation.—Add to a warm solution of chondrin in water, † 1. Acetic acid. It will be precipated. † 2. Add to this a little sodium chloride or sulphate. The precipitate will redissolve. 3. Add sodium sulphate to a watery solution of chondrin, and afterwards acetic acid. No precipitate will fall. 4. Dilute hydrochloric or other mineral acid. The chondrin is precipitated and is dissolved by excess of acid. 5. Alum precipitates chondrin; excess dissolves it. 6. Lead acetate, 7, Silver nitrate, 8, Chlorine water, all precipitate chondrin.

Effect of Boiling.—Boil a watery solution of chondrin for a long time. Let it cool, and it will be found to have lost its power of gelatinizing, but it will give the other reactions just as before.

Decomposition of Chondrin.—By boiling with concentrated hydrochloric acid, chondrin is decomposed, and yields grape sugar, and certain nitrogenous substances. The presence of grape sugar may be tested by the reactions given in § 77 or § 155.

51. Distinctive Characters of Mucin, Chondrin, Gelatin, and Albumin.

Mucin.—Precipitated by acetic acid, the precipitate is not dissolved by sodium sulphate.

Chondrin.—Precipitated by acetic acid, the precipitate is dissolved by sodium sulphate.

Gelatin.—Not precipitated by acetic acid, nor by acetic acid and potassium ferrocyanide.

Albumin.—Dissolved by acetic acid, the solution is precipitated by potassium ferrocyanide, or by the addition of alkaline salts and heat.

Gelatin and Chondrin are most generally recognized by their hot solutions forming a jelly on cooling; but as they are both deprived of this property by long boiling or boiling with acids, this test is not always to be depended on.

** 52. **Bone.**—When bone is subjected to the action of acids, the earthy salts are removed. The remainder, to which the name ossein has been given, consists chiefly of gelatigenous substance. The earthy salts are tribasic calcium, and magnesium phosphates, calcium carbonate, and small quantities of calcium fluoride.

To remove the earthy salts, and leave the ossein, place a bone for some time at a low temperature in very dilute hydrochloric acid. When treated with warm dilute hydrochloric acid, bone gives out CO_2 and is apt to separate into lamellæ. The ossein is soft, flexible, and elastic while moist, but becomes hard when dry. It retains the form of the bone. In its chemical characters it resembles the gelatigenous substance from connective tissue.

To get the earthy salts, incinerate the bone, when the organic substance will be consumed, and they will remain behind, mixed with other salts formed during the combustion, for here as in other cases the salts in the ash differ considerably from those which exist in the tissue.

** 53. **Adipose Tissue.—Fats.**—Fats differ from each other in appearance and consistence. Their general properties may be conveniently studied in olive oil, for which cod liver oil or train oil may be substituted, if an animal fat is desired.

Solubility.—Fats are insoluble in 1. Water, and 2. Cold alcohol. † 3. Hot alcohol. Warm a test-tube containing oil and alcohol over a spirit-lamp or Bunsen's burner. As the spirit becomes warm, part of the oil will be dissolved. Pour off some of the clear alcoholic solution into another tube and cool it. It will become milky from the deposition of oil. † 4. Cold ether. Shake a little oil with ether and it will dissolve readily. The test-tube containing the ether must not be brought near a flame, as its vapour is readily inflammable. 5. Chloroform; 6. Oil of turpentine, and other volatile oils, also dissolve fat readily.

* *Emulsionizing of Fats.*—Shake a little oil with a solution of albumin in a test-tube. The oil will become finely divided, and form a milky-looking fluid or emulsion. Put a drop of this under the microscope, and it will be found to consist of minute globules of fat. The globules in the emulsion unite again and form large globules, but very slowly. Add a little acetic acid to the emulsion and shake it. The globules will unite much more quickly. Repeat the experiment with a solution of gelatin. This also will emulsionize the fat.

Reaction.—Wash a piece of lard in water and press a piece of litmus paper against it, or melt it in a test-tube and put a drop of it or of olive oil on the paper. Its reaction will be neutral..

Composition of Fats.—Fat consists of a triatomic radicle, propenyl or glyceryl, combined with three atoms of a monatomic fatty acid. The glyceryl may be displaced by inorganic bases, such as potassium, lead, &c., and glyceryl hydrate, or glyceryl alcohol (glycerin) is produced. The replacement of glycerin by other bases is termed saponification.

Boil two and a half grammes of olive oil with one gramme of very finely powdered lead oxide, and about fifty cubic centimeters of water in a beaker or evaporating dish for some hours, stirring the mixture well to prevent the lead oxide from falling to the bottom, and replacing the water as it evaporates. The lead will combine with the fatty acid in the oil, forming a slightly yellowish mass, and the glycerin will be set free. To obtain the glycerin, filter the fluid; pass sulphuretted hydrogen through the filtrate, add a little animal charcoal to decolorize it; let it stand for a while in a warm place; filter and evaporate the filtrate.

54. Glycerin.—Glycerin is a syrupy fluid, with a sweet taste and a neutral reaction.

Solubility.—1. With water, and, 2, with alcohol, it mixes very readily. 3. With ether it does not.

Solvent Power.—It dissolves many metallic oxides. Add a little liquor potassæ to a solution of copper sulphate or lead acetate, a precipitate will fall. Add a little glycerin, and the precipitate will redissolve.

It also acts to some extent as a solvent for fatty acids.

Decomposition.—Put a little glycerin, free from water, into a test-tube, with glacial phosphoric acid or acid potassium sulphate, and heat. The glycerin will be decomposed, and yield water and acrolein or acrol, a body which has an extremely unpleasant smell, and causes great irritation of the nose and eyes.

Test for Glycerin.—As no other body yields acrolein when decomposed in the way just mentioned, its formation serves as a test for glycerin; and as it is very pungent, small quantities of glycerin can easily be detected.

55. **Muscle.**—For the structure of muscle, *see* Chap. IV.

Reaction.—Muscles which have been at rest have an amphichromatic reaction; *i.e.*, they change red litmus to blue, and also blue litmus to red. They do not alter the colour of blue litmus so much as that of red, and they are therefore alkaline.

Alteration in the Reaction by Contraction.—The reaction changes to acid after contraction of the muscle or after death. *See* Chap. XX., *Obs.* VI.

56. **Composition of Muscle.**—**The Sarcolemma** is usually said to agree with elastic tissue in its characters, and to yield no gelatin, but it has been recently stated to be soluble, though slowly, in alkalies and acids, as well as in gastric juice, and would thus more nearly resemble connective tissue.

57. **Sarcous Elements.**—Little is known regarding the chemical composition of the sarcous elements, except that they swell slightly, and lose their power of double refraction when boiled or when heated with alkalies or very dilute acids. Alcohol does not alter them.

† 58. **Muscle Plasma.**—When muscles are subjected to pressure at 0° C., a fluid termed muscle plasma is obtained. The plasma of muscles resembles the plasma of the blood, in possessing the power of coagulating spontaneously, and separating into a clot and serum. To this clot, corresponding to the fibrin of the blood, the name myosin has been given. Coagulation of the plasma causes the muscles to lose their elasticity and become stiff and hard, and thus gives rise to *rigor mortis*. After some time, decomposition sets in, and the muscles again become soft and flexible. Muscle plasma is somewhat troublesome to obtain, as it coagulates too quickly in the muscles of warm-blooded animals to allow of its preparation from them, and the muscles of frogs, in which it coagulates more slowly, are not always to be had in sufficient quantity.

Preparation.—Prepare a freezing mixture by mixing together equal parts of salt and snow, or pounded ice. Introduce it into a large beaker, and plunge a platinum crucible or small tin box into it. Fill another beaker with half-per-cent. salt solution, and put it in a vessel containing snow or ice. Prepare several frogs in the following manner:—Open the thorax, cut off the apex of the heart, push a cannula up into the aortic bulb, and inject a half-per-cent. salt solution through it, in the manner directed for artificial circulation in Chap. XVI. § 45, till the fluid which issues from the veins is quite colourless. Cut away the muscles close to their attachments, and wash them with half-per-cent. salt solution cooled to 0° C. When washed, squeeze them tightly together into a ball, and tie them up in a piece of thin linen; put them into the crucible or tin box. As the muscles of each frog are prepared add them to those in the crucible, and let it remain in the freezing mixture until they are all frozen quite hard. Take a sharp knife and cool it in the freezing mixture; cut the frozen mass of muscle into very thin slices; throw them into a mortar cooled in the same way, and break them up small. Tie them up in a piece of strong linen, and put them into a strong screw-press. As the temperature of the muscle is gradually raised by the warmth of the room to 0° C., the frozen plasma melts and issues from the press. It must be collected in a vessel cooled in ice, and filtered through paper moistened with cold half-per-cent. salt solution, and collected in a cold beaker. The funnel may be kept cold during filtration by placing it in a double copper filtering stand of the form shown in fig. 336, but filled with snow or pounded ice, instead of hot water. As the filters get soon choked, they must be frequently renewed. The filtered plasma is a slightly yellowish and opalescent, syrupy, but not tenacious, fluid.

Reaction.—Its reaction is alkaline, like that of muscle.

Coagulation of Muscle Plasma.—Transfer a little plasma from the beaker to cooled test—tubes and observe the following facts :—

It will coagulate spontaneously when allowed to stand at the temperature of the room, and form a gelatinous clot, which will begin at the sides of the tube, and extend inwards.

By stirring, a coagulum is obtained, which is flocculent, and not fibrous like the fibrine of blood.

Heat greatly accelerates its coagulation, and at 40° C. it coagulates almost instantaneously.

Cold water coagulates it at once, so that the plasma when dropped into it forms white elastic balls. Cold NaCl solution of fifteen per cent. also coagulates it, but a solution of five per cent. does not. Dilute hydrochloric acid of ten per cent. coagulates it at once, but dissolves the coagulum, and forms syntonin almost immediately.

** 59. **Examination of the Aqueous Extract of Muscle.**—In order to obtain an aqueous extract of muscle, a dog must be killed by decapitation, and the blood removed from the vessels of the lower extremities by artificial circulation. For this purpose open the abdomen quickly, and insert a cannula in the aorta. Inject ten-per-cent. NaCl solution into it till the blood returns colourless by the *vena cava*. Cut off some of the muscles of the thigh quickly, and mince them up small. This is best done by a sausage-making machine. Mix the mass with distilled water, stir it up well, and let it stand for a quarter of an hour. Filter it through linen, aiding its filtration by pressure.

** 60. **Albuminous Substances in Muscle.—Alkali Albuminate.**—The watery extract thus obtained contains alkali albuminate. It is at first alkaline or neutral, but afterwards becomes acid, and the alkali albuminate is then thrown down as a flocculent precipitate. The source of the acid is not known. If the extract has been made from muscle which has already become acid, this precipitate will not fall.

To a portion of the extract add dilute hydrochloric, acetic, or lactic acid very gradually. A flocculent precipitate will fall.

Repeat the last experiment, using exactly the same quantities of extract and acid, but add a little sodium phosphate to the extract before acidulating it. No precipitate will fall. *See* § 15.

Albumins.—Besides alkali albuminate, the extract contains two other albuminous substances, one of which coagulates at 45° C, the other at 75° C. Filter the fluid from which the alkali albuminate has been precipitated either by the development or the addition of acid. Put some in a test-tube and warm it in a water bath to 45° C. A precipitate will form. The coagulation is not affected at all by previously rendering the liquid neutral or alkaline. Let the fluid stand till the precipitate subsides, and then remove it by filtration, and warm the filtrate to 70° C. A second coagulation will take place.

** 61. **Myosin.**—Free the remainder of the muscles from fascia, tendons, fat, nerves, and vessels, and cut them up small. Put the mass of finely-divided muscle into five or six times its weight of water and stir it well. Let it stand for several hours and then strain it through a linen cloth, and express the fluid with the aid of a screw-press. Treat the muscles a second time with water in the same way, and strain and press again. Unite all the fluids thus obtained and keep them for examination. Wash the muscle which remains on the linen with water as before, till it becomes of a greyish colour, and the water is no longer coloured.

Throw it into a mortar, and rub it up with ten-per-cent. salt solution in sufficient quantity to prevent it from being too thick and to allow it to flow tolerably easily. Let it stand for several hours; filter, first through linen, then through paper, and add to the filtrate several pieces of rock salt. As the salt dissolves, the myosin, which is insoluble in a concentrated NaCl solution is precipitated in flocculi. If any salt remains undissolved after the myosin seems fully precipitated, remove it, and then filter the solution. The myosin which contains a large amount of NaCl remains on the filter. In order to free it from this, dry it as well as possible by pressing it between folds of filtering paper; dissolve it in a little water, and throw the solution into a large beaker full of water, when it will again be precipitated. Let it stand for a day, pour off the clear fluid as well as possible, and then collect the precipitate on a filter. After the greater part of the water has passed through the filter, but while the precipitate is still moist, remove it into a beaker, as it cannot be separated from the filter after it becomes dry.

Solubility.—Test the solubility of the moist myosin in the following reagents: † 1. Ten-per-cent. NaCl solution. The myosin will dissolve. Put some sodium chloride in substance into the solution. As it dissolves, and the solution becomes saturated, the myosin will be precipitated. It is soluble in 2. Solution of sodium sulphate or other neutral salt; 3. Very dilute liquor potassæ; and 4. Very dilute hydrochloric acid.

Action of Acids and Alkalies.—Dilute acid and alkalies dissolve myosine, as has just been seen. At first it is simply dissolved, but is very soon converted into acid albumin or alkali albuminate. Divide the solutions of myosin in dilute liquor potassæ and dilute hydrochloric acid just made, into two portions, add salt solution immediately to one portion of each, put in a drop of litmus, and neutralize both. No precipitate will fall, for the myosin being unchanged is soluble in the salt solution. Let the other portions stand for ten minutes, and then treat them in the same way. A precipitate will fall on neutralizing them, for the myosin, being now converted into alkalial buminate and syntonin, is no longer soluble in NaCl solution.

Coagulation of Myosin.—1. Boil a NaCl solution of myosin; it will coagulate. 2. Add alcohol to its NaCl solution, and a similar coagulum will form.

Effect of Drying.—Dried myosin is tough and difficult to powder, and almost insoluble in NaCl solution.

** 62. **Extractive Matters in Muscle.**—The cold watery extract of muscle contains, beside the albuminous matters, creatine, creatinine, hypoxanthine (sarkin), xanthine, uric acid, inosic acid (apparently not always present), glucose, inosite, salts of lactic acid, and volatile fatty acids and acid phosphates of the alkalies. Unless a large quantity of muscle can be got, it will be better to use Liebig's extract for the preparation of these substances. Put the watery extract of muscle in a tin kettle; heat it quickly to boiling, so as to coagulate the albumin. Filter it through a linen cloth. Let the filtrate become quite cool, and add acetate of lead to it as long as a precipitate is formed. Excess of lead must be avoided as much as possible. Collect the precipitate on a filter, and keep it for after examination. . . . . . (*a*)

63. **Creatine.**—Precipitate any lead present in the filtrate by hydrogen sulphide; filter; evaporate the filtrate to a thin syrup on the water-bath. Put it in a cool place for several days, and the creatine will separate in short colourless crystals. Let it stand till no more crystals are deposited; pour off the mother liquor from the crystals, and add to it two or three times its volume of alcohol of 88 per cent., so as to cause the suspended creatine to be deposited. Filter it, and wash the crystals with a little alcohol. Wash off the crystals which still remain on the evaporating dish with the alcohol which drops from the filter, throw them also on the filter and wash them with a little alcohol. Collect the filtrates, mix them, and put them aside. . . . . . (*b*)

Dissolve the crystals in a little boiling water, and allow the solution to cool, when the creatine will crystallize out in colourless transparent and lustrous oblique rhombic prisms, which, when gently heated on a piece of platinum foil, lose water of crystallization, and become dull and whitish.

Solubility.—Creatine is sparingly soluble in cold water; easily soluble in boiling water; almost insoluble in strong alcohol; insoluble in ether.

Reaction.—The solution in hot water has a neutral reaction, and bitter taste.

Test.—Creatine has no very characteristic reactions, and it is best recognized by converting it into creatinine. If it is pure, no precipitate will fall on the addition of zinc chloride to its solutions, but if mixed with creatinine a precipitate will be produced.

Decomposition.—When it is boiled for a considerable time with caustic baryta, creatine decomposes into urea and sarcosin. If the boiling is continued still longer, the urea decomposes into carbonic acid and ammonia. This reaction is very interesting as indicating one source of urea in the body. When boiled with water for a long time or with acids, it loses water and is converted into creatinine.

64. *Creatinine.*—Boil creatine for half an hour with dilute hydrochloric acid; neutralize with hydrated lead oxide; filter; evaporate the filtrate to dryness on the water-bath. Extract the residue with alcohol, and evaporate the extract. The creatinine will crystallize in colourless lustrous prisms, which, when heated on platinum foil, do not dry like creatine.

Solubility.—It is soluble in water, especially when hot. Unlike creatine, it is soluble in hot alcohol.

Reaction.—Test the watery solution with litmus or turmeric paper; it will be found strongly alkaline. It has a taste like dilute ammonia.

Characters.—Creatinine acts like a strong alkali, and forms double salts with metals. The most important is its compound with zinc chloride. Add to an alcoholic or not very dilute watery solution of creatine, a concentrated syrupy solution of zinc chloride free from hydrochloric acid; a precipitate of warty granules will fall at once if the solution is concentrated; but if dilute, groups of needles will slowly form. The granules are seen under the microscope to consist of radiating groups of fine needles. They are very sparingly soluble in cold water; more so in hot; insoluble in alcohol; but very soluble in mineral acids.

This test is sufficient to distinguish creatinine. It is further precipitated by silver nitrate, by mercuric chloride, and by mercuric nitrate with the gradual addition of sodium carbonate.

65. **Sarkin (Hypoxanthine).**—Evaporate the alcohol from the filtrate (*b*) upon the water-bath; dilute it with water; render it alkaline by ammonia, and then add an ammoniacal solution of silver nitrate. Sarkin will be precipitated. Let the flocculent precipitate subside; wash it several times by decantation with water containing ammonia; throw it on a smooth porous filter, and wash it thoroughly; push a glass rod through the bottom of the filter, and wash the precipitate with nitric acid of 1·100 sp. gr. into a small flask. Heat it to boiling, and add more nitric acid till the whole is dissolved. The fluid should be kept nearly boiling. Sometimes a few flakes of silver chloride remain undissolved. Decant the liquid from them into a beaker, and let it stand for six hours. A double nitrate of silver and hypoxanthine will crystallize out.

Decant the liquid (*c*) from the crystals and preserve it for the preparation of xanthine. Wash them with an ammoniacal solution of silver nitrate to remove the free acid. Suspend them in water, and pass hydrogen sulphide through it. Filter from the silver sulphide, and evaporate the filtrate. The hypoxanthine will crystallize out.

In its reactions it resembles xanthine, but differs from it in being precipitated by silver nitrate.

66. **Xanthine.**—To the mother liquor (*c*) of the hypoxanthine add ammonia in excess. A flocculent precipitate of nitrate of silver and xanthine will fall. Wash it by decantation; suspend it in boiling water, and decompose it by hydrogen sulphide. Filter and evaporate. The xanthine will separate as a scaly film.

Tests.—Put a little xanthine in ammonia. It will dissolve. Add a little nitric acid to a portion of xanthine in a porcelain capsule; evaporate to dryness. A yellow residue will remain. Add a drop of caustic soda to it, and it will become red. Heat it, and the colour will change to purple red.

Put liquor sodæ in a watch-glass with a little chloride of lime; stir it, and introduce a portion of xanthine. A ring will form round it, at first dark green, but soon becoming brown, and then disappearing.

67 **Uric Acid.**—Suspend the lead precipitate (*a*) in water; decompose it completely by hydrogen-sulphide; filter; concentrate the filtrate in a water-bath. Uric acid will separate gradually.

Filter, and set the filtrate aside (*d*). Wash the crystals on the filter with a little water and then with alcohol. If desired, they may be further purified by dissolving them in a little liquor sodæ, precipitating by ammonium chloride; filtering and decomposing by dilute hydrochloric acid.

Murexide Test.—Put a small portion of uric acid on a watch-glass, with one or two drops of nitric acid, and evaporate to dryness at a moderate temperature. A yellow residue will remain, which becomes red when quite dry. Put a drop of ammonia on the side of the glass, and let it run gently down to the uric acid, which will then become of a beautiful purple. If a drop of liquor potassæ or liquor sodæ is used instead of ammonia, a bluish-violet colour will be produced.

Inosite.—Evaporate the filtrate (*d*) till a permanent turbidity is produced by the addition of alcohol. Then add its own volume of alcohol to it and warm it, when the turbidity will disappear. Set it aside for several days. Inosite may then crystallize out. If it does not, add ether; and if still no crystals form, evaporate almost to dryness; add a little nitric acid, evaporate to dryness; moisten it with calcium chloride, and evaporate to dryness again. If inosite is present, a rosy red spot will remain.

If crystals have been formed, dissolve some in water, in which they are easily soluble, and apply the same test.

68. **Brain.**—The brain contains cholesterin, lecithin, and cerebrin, besides albuminous substances, which chiefly form the axis

cylinders, and are insoluble in water. Cerebrin probably belongs to the white substance of nerves.

The specific gravity of the brain may be ascertained in the manner directed in App. § 216, and the amount of water it contains by weighing it, drying it in a hot chamber, or over sulphuric acid, and estimating the loss. To separate the substances contained in the brain, remove the membranes and vessels as much as possible from it, wash its surface with water, and rub it to a paste in a mortar. Mix it with great excess of alcohol, and let it stand for several days, stirring it frequently. Separate the alcohol by filtration, and set it aside for the preparation of lecithin. . . . . (*a*)

Rub up the brain again, and extract it with large quantities of ether, as long as they take up much lecithin or cholesterin. This is known by evaporating a small quantity of the ether each time it is taken from the brain. Put the ether aside; extract the brain with hot alcohol several times, and filter it hot. On cooling, cerebrin will crystallize out, mixed with lecithin.

69. Cerebrin.—*Purification.*—Filter off the alcohol from the crystals, wash them with cold ether, and boil them for an hour with baryta water. Pass CO_2 through the liquid to precipitate the excess of baryta; filter, and wash the precipitate first with cold water and then with cold alcohol. Put the precipitate in a beaker with alcohol and heat it, to extract the cerebrin from it, and filter it hot. On cooling, crystals of cerebrin will be deposited, which should be again dissolved in hot alcohol, allowed to crystallize out again, washed with ether, and dried at a moderate temperature.

Cerebrin forms a white hygroscopic powder. Put a little on a piece of platinum foil and heat it. It will become brown, melt, and then burn.

From the mode of preparation, it is evident that it is insoluble in cold but soluble in hot alcohol, and that it is not destroyed by boiling with baryta water.

Put it in water. It will slowly swell up, somewhat like starch.

70. Lecithin.—Add to the alcoholic extract (*a*) a solution of platinum chloride, acidified with hydrochloric acid. A yellow flocculent precipitate of lecithin platinum chloride will fall. Filter, and dissolve the precipitate in ether; pass hydrogen sulphide through the solution to precipitate the platinum. Filter and evaporate. Lecithin chloride will remain as a waxy mass.

Decomposition.—When treated with acids or with boiling baryta water it is decomposed, and yields glycerophosphoric acid, neurin, and fatty acids.

Dissolve some lecithin chloride in alcohol and pour it into boiling baryta water. It will be decomposed, and a smeary precipitate will fall.

71. **Neurin.**—Filter; pass CO_2 through the filtrate to remove the baryta; filter; evaporate to dryness; extract with alcohol. Add to the alcoholic extract platinum chloride, and a precipitate of neurin platinum chloride will fall. The platinum may be removed by hydrogen sulphide and the neurin chloride obtained, but it is with difficulty crystallizable.

CHAPTER XXXVII.

DIGESTION.

SECTION I.—SALIVA AND ITS SECRETIONS.

72. Mode of obtaining Mixed Saliva.—To obtain a sufficient quantity of human saliva for examination, the secretion of the salivary glands must be stimulated artificially. For this purpose any of the mechanical or chemical stimuli to be mentioned in § 85 may be used. To avoid the risk of the saliva becoming altered by mixture with the substance used to quicken its secretion, the mechanical stimuli should be preferred. There is no objection, however, to the employment of ether vapour.

** **73. Examination of Mixed Saliva.**—*Appearance.*—Saliva is transparent or opalescent. It sometimes deposits a white precipitate almost immediately after it has been collected. When poured from one vessel to another, it is seen to be more or less viscid, in consequence of which it is generally filled with air-bubbles. If none are present, they are readily produced by blowing into the liquid through a narrow glass tube, when it is seen that they take a long time to subside. If the saliva is allowed to stand long, a thin pellicle of carbonate of lime forms on its surface. *Microscopical Examination.*—Saliva contains numerous air-bubbles, pavement epithelium cells from the mouth, and round cells (salivary corpuscles) resembling lymph corpuscles, within which are numerous granules in constant movement.

** **74. Determination of the Amount of Water and of Solids.**—Take a small porcelain crucible with a lid, dry it in an air-bath at 100° C., put it under a bell-jar over a dish containing strong sulphuric acid till it is quite cool, then weigh it immediately and note its weight carefully. After weighing it, replace it in the air-bath for another hour, cool it and weigh it again as before. If the weight is less the second time than the first, the process must be repeated till no further loss of weight occurs. Introduce some saliva into it and weigh again.

The amount of saliva used is ascertained by deducting the weight of the crucible alone from the weight of the crucible and its contents, thus :—

Weight of crucible and saliva	33·462 grm.
Weight of crucible alone	23·296 grm.
	10·266 = weight of saliva used.

Evaporate the saliva to dryness either in the air-bath or over a water-bath, but finish the desiccation in the air-bath. Cool and weigh the crucible as before. The amount of solid residue is determined in the same way as that of the saliva itself, thus :—

Weight of crucible and dried residue	23·342 grm.
Weight of crucible alone	23·296 grm.
Difference	·046 grm. = weight of residue.

The amount of water is found by subtracting the weight of the solid residue from that of the saliva used, thus :—

Weight of saliva used	10·266
Weight of solid residue	·046
	10·220 weight of water.

$$\text{Hence percentage of water} = \frac{10{\cdot}220 \times 100}{10{\cdot}266} = 99{\cdot}5 \text{ and}$$

$$\text{Percentage of solid residue} = \frac{0{\cdot}046 \times 100}{10{\cdot}266} = 0{\cdot}44$$

* 75. **Qualitative Investigation of Inorganic Constituents.**—For this purpose the saliva must be filtered so as to separate the epithelium and mucus. It contains carbonates, chlorides, phosphates and sulphates of potassium, sodium, calcium, and magnesium, and in most cases also potassium sulphocyanide. The presence of these several salts may be demonstrated as follows: *Carbonates.*—If a drop of saliva is placed on an object-glass and covered in the usual way, and a drop of acetic acid added, bubbles of gas will be seen to form under the cover-glass. *Chlorides.*—The saliva is acidulated strongly with nitric acid, after which solution of silver nitrate is added; the precipitate formed is insoluble in excess of acid, but dissolves readily in ammonia. *Sulphates.*—The turbidity produced by solution of barium, chloride, or nitrate does not disappear when nitric acid is added, and the liquid is boiled. *Potassium.*—If a little saliva is gently evaporated on a platinum wire and then heated in the flame of a Bunsen's lamp, the flame seen through blue glass exhibits a violet colour. *Sodium.*—Without the glass it presents the well-known yellow colour due to the presence of

sodium. *Calcium* may be precipitated as oxalate by the addition of ammonium oxalate, *Magnesium* as ammoniaco-magnesian phosphate. To obtain the latter, ammonium chloride, and ammonia must first be added, then sodium phosphate. *Potassium Sulphocyanide.* — This is generally, though not invariably present in mixed saliva. It is derived from the saliva secreted by the parotid gland, and is not contained in that of the submaxillary gland. To show its presence, add a drop of solution of perchloride of iron, so very dilute as to be almost colourless, to a little saliva in a porcelain crucible or capsule, and stir it. A reddish colour is developed, which remains unchanged after the addition of hydrochloric acid, but is at once removed by a solution of corrosive sublimate. Perchloride of iron gives a similar colour with acetic acid and with meconic acid, but the colour produced in the former case is destroyed by hydrochloric acid and in the latter by mercuric chloride. When undiluted perchloride of iron is used, the colour is deep red, and may be shown to persons at a little distance. If the test does not at first succeed, the saliva should be evaporated to one-third of its bulk, and the test then applied.

To determine the *percentage of inorganic salts*, the dry residue must be incinerated (*see* § 214), weighed, and calculated, as in § 74.

* 76. **Organic Constituents.**—These are albumin, mucin, ptyalin. *Albumin.*—If saliva is strongly acidified with nitric acid, it becomes turbid, but no precipitate is formed. On then boiling it becomes clearer, and the colour changes to yellow; the addition of ammonia changes the yellow to orange-red. If to another portion a mixture of acetic acid and potassium ferrocyanide is added, a white precipitate is produced. Saliva contains two albuminous bodies—albumin proper dissolved in salts, and *globulin.* Globulin is precipitated from dilute solutions by CO_2, ordinary albumin is not. To separate them, a stream of carbonic acid gas must be passed through saliva, diluted with a large quantity of water, for some time. A very fine flocculent precipitate is formed, which tends to disappear when the turbid liquid is agitated with air. After the precipitate has settled, the liquid may be decanted off with a syphon, and if needful, filtered; it can then be proved to contain albumin by the addition of acetic acid and ferrocyanide of potassium. This process requires considerable care. *Mucin.*—To this body is due the stickiness and tenacity of saliva. If acetic acid is gradually added to saliva while it is stirred with a glass rod, it becomes more and more tenacious, and finally the mucin separates in white stringy flakes; these must be washed with water and acetic acid, and tested by the reactions given in § 45.

** 77. **Action of Saliva on Starch Paste.**—Saliva converts starch into sugar. To show this prepare some thin mucilage by rubbing up a little starch with cold water into a smooth paste and pouring a large quantity of boiling water over it (one grain of starch to one hundred centimeters of water), or by boiling it in a flask or large test-tube and then allowing it to cool. Filter the saliva to be used, and distribute it in three test-tubes, introducing into the first, starch mucilage alone—into the second, saliva—and into the third, saliva with about three times its bulk of starch paste. Mix them well together by agitation. Then put all three for a few minutes into a water-bath at 40° C., or warm them gently over a spirit-lamp. Add to each of them liquor potassæ in excess, and a drop or two of solution of cupric sulphate. In the first and second, a light blue precipitate will be thrown down, and the liquid will remain colourless; but in the third, the precipitate just formed will be redissolved, and give a blue solution. If now the liquids are boiled, the precipitate in the first tube, containing starch paste, alone will be blackened, but the liquid will remain colourless. In the second, containing saliva, the precipitate will be partly dissolved, and give to the fluid a violet colour, due to albumin in the saliva, § 12. In the third, a yellow or orange precipitate will be formed. This reaction, which is known as Trommer's test, shows that there is no sugar either in the saliva or starch used, but that it is formed by the action of the one on the other. *Rapidity of conversion of starch into sugar.*—Bidder and Schmidt erroneously considered that the conversion of starch into sugar was almost instantaneous. To illustrate this view, introduce saliva into a small beaker. Place it in a water-bath at 40° C., and when it is warmed through, let a little dilute starch mucilage, coloured with iodine, fall into it drop by drop. As each drop falls it becomes decolorized. The disappearance of the blue colour is not dependent on the conversion of starch into sugar, but on the conversion of the iodine into hydriodic acid. Other organic fluids, such as the urine of dogs, according to Schiff, exhibit the same reaction, which is probably due to their containing deoxidizing substances, for the same effect is produced by sulphurous acid or morphia, both of which absorb oxygen readily. This may be shown by putting starch mucilage coloured with a little iodine into a test-tube and diluting it till it forms a clear blue transparent solution. If it is now placed in the warm bath at 40° C., it will remain unaltered, but will at once lose its colour on the addition of either of the reducing agents above mentioned.

* 78. **Effect of Temperature on the Diastatic Action of Saliva.**—Take four test-tubes, and carefully introduce a little saliva into each with a pipette. Put the first into a mixture of snow or ice and salt, the second into a test-tube rack on the table, the third into a water-bath at 40° C.; boil the fourth briskly for two or three minutes, and then allow it to cool. Then add starch paste to each of them, and allow them to remain where they are for five or ten

minutes. Take a part of the fluid from each, and test it for sugar, either by Trommer's or Moore's tests. (*See* § 155.) None will be found in the first or fourth, a little in the second, and more in the third. Thus we learn that saliva does not act, or acts very slowly, at the freezing point, that it acts at the temperature of the air, and still more quickly at the temperature of the body. Now place the first and fourth test-tubes in the water-bath at 40° C., allow them to remain for several minutes, and test again for sugar. It will be found in the first but not in the fourth. This shows that the power of saliva to transform starch into sugar is merely suspended by exposure to a very low temperature, but is totally destroyed by boiling.

* 79. **Influence of Acids and Alkalies on the Diastatic Action of Saliva.**—Dilute *acids* do not arrest the action of saliva upon starch; stronger acids do so for a time, but when they are neutralized the action again goes on.

Take three test-tubes, and put into each equal parts of saliva and starch paste. Add to the first its own bulk of water, to the second a similar proportion of distilled water, containing 0·65 per cent. of *commercial* hydrochloric acid, and to the third the same quantity of dilute acid of 10 per cent., and keep them for five minutes at 40° C. Add liquor potassæ to the first and second, and test for sugar. It will be found in nearly equal quantity in both. Take part of the fluid in the third tube, and test it for sugar. None will be found. Neutralize the remainder with carbonate of potash, carefully avoiding excess, and replace the test-tube in the water-bath for a little while. On again testing it, sugar will be found to be present. —As the greater part of the starch we eat is not transformed into sugar in the mouth, but is swallowed unchanged, it is important for us to know whether the transformation goes on in the stomach or whether it is arrested by the acid gastric juice. The strength of the dilute acid just employed (0·2 of *real* hydrochloric acid) is nearly the same as that of the gastric juice, and the experiment shows that in the healthy stomach the conversion of starch into sugar may go on rapidly. In some pathological conditions the acidity of the gastric juice is abnormally increased, and the action of the saliva may be suspended so long as the food remains in the stomach, but when the acid is neutralized by the intestinal secretion, the action will go on again.

Alkalies.—Caustic potash and soda, when added to the saliva in excess, put a stop to its action on starch, and its diastatic power is not restored by neutralization. Its action is suspended by sodium and potassium carbonates, ammonia and lime water, but restored by neutralization. Put saliva in two test-tubes and add to one several drops of liquor potassæ, and to the other a few drops of a solution of potassium carbonate, mix a little starch mucilage with both, and let them stand in a water-bath at 40° C. for half an hour. Test a small portion of the liquid from both tubes, and having ascertained that neither contains sugar, put a drop of litmus solution in each, and neutralize with dilute hydrochloric acid. After both have stood for another half hour, sugar will be found in the one to which the carbonate was added, but not in the other.

* 80. **Action of Saliva on Raw Starch.**—As has been seen, the saliva rapidly converts starch mucilage into sugar, but it does not act so quickly on raw starch. The starch granules consist of a number of layers arranged in an eccentric manner round a point called the hilum. These layers consist alternately of two substances which have been termed respectively, starch-cellulose and starch-granulose. The latter is coloured blue by iodine alone; the former is not coloured unless the granules have been previously acted on by sulphuric acid or zinc chloride. When starch is digested with saliva, the granulose only is dissolved, and although the starch granules still retain their form, they are no longer coloured blue by iodine.

To show this, potato starch must be mixed with saliva, and subjected for two or three days to a temperature of 35° C. The saliva used must be decanted off, and a fresh quantity added every two or three hours. The starch is prepared for the purpose by placing a quantity of the pulp obtained by scraping the cut surface of a raw potato on a bit of calico stretched over the mouth of a beaker, and then washing it with a gentle stream of water. The starch granules pass through into the beaker, leaving a fibrous residue on the calico.

81. **Artificial Saliva.**—As ptyalin is present, ready formed, in the salivary glands, a fluid which, like saliva, will convert starch into sugar, can be obtained by making an infusion of the glands.

Take the salivary glands of an ox, sheep, rabbit, or guineapig. Remove the cellular tissue from them, chop them up fine, and let them stand with a little water upon them for several hours. Strain through muslin and filter. The filtrate may be used instead of saliva for the experiments already described.

* 82. **Preparation of Ptyalin from the Salivary Glands.**—Ptyalin may be separated from the infusion of the glands in the same manner as from saliva, but as it dissolves very readily in glycerin, it is much more advantageous to extract it by that agent. For this purpose prepare the salivary glands of an ox or sheep, as above directed. Place the well-minced gland in a flask, and cover it with absolute alcohol. Cork the mouth of the flask, and let it stand for twenty-four hours. Then, having poured off the liquid, squeeze the remainder in a cloth, so as to get rid of as much of the alcoholic extract as possible. The cake so obtained must now be

mixed with as much glycerin as will cover it in a beaker, and allowed to remain for several days, during which the mixture may be occasionally stirred. At the end of this period, the whole must be strained through muslin, and then filtered through paper. In the filtrate, ptyalin is precipitated by the addition of alcohol in excess. The precipitate, after having been collected by subsidence and decantation, must be dried over sulphuric acid.

83. **Separation of Ptyalin from Saliva.**—The method employed for separating ptyalin as well as other ferments from the secretions in which they are contained, depends on the fact that when a copious precipitate is produced in the fluid, the ferment adheres to the particles of the precipitate, and is carried down along with them. It does not, however, adhere very closely to the precipitate, and can readily be washed off. The precipitate employed to carry down ptyalin is calcium phosphate. This carries down with it not only the ptyalin, but also the albumin in the saliva. The albumin, however, adheres more closely than the ptyalin to the precipitate, so that the ptyalin is dissolved away by the first wash-water, while the albumin remains adherent. Collect a considerable quantity of saliva by filling the mouth with ether; while fresh, acidify it strongly with phosphoric acid, so that the precipitate to be produced may be voluminous; then add milk of lime till the fluid has a faintly alkaline reaction, and filter. When the fluid has drained from the precipitate, remove the latter into a fresh beaker, add to it a little water, not exceeding in amount the saliva originally employed, stir it well and filter again. Add to the filtrate an excess of alcohol. After some time a fine white flocculent precipitate will separate, which must be collected in a filter and dried over sulphuric acid. It then forms a snow-white powder, and consists of ptyalin mixed with some inorganic salts. To obtain it free from ash, dissolve it in water, and precipitate it again by absolute alcohol. Pour off the alcohol, dissolve again in water, and precipitate again. Repeat this several times, collect the precipitate on a filter, wash with dilute alcohol, and then with a little water, and finally dry it at a low temperature, under a bell-jar over sulphuric acid.

* 84. **Properties of Ptyalin.**—The reactions of ptyalin may be examined either in the filtered aqueous solution of the calcium phosphate precipitate, or in solutions of pure ptyalin. Ptyalin differs entirely from albumin in its reactions.

1. Add nitric acid; there is no precipitate. Boil the liquid, allow it to cool, and add ammonia. No yellow colour is produced.

2. Add to several portions in test-tubes, mercuric chloride; tannic acid; acetic acid and solution of potassium ferrocyanide; platinum chloride; solution of iodine. No precipitate appears in any case, but the iodine produces a yellow colour.

3. Add lead acetate, and to another quantity basic lead acetate.

In both cases a precipitate is formed after a time, and on filtration it is found that the filtrate is without action on starch, the ptyalin having been carried down with the precipitate.

4. Add liquor potassæ and cupric sulphate. No reduction of the copper oxide occurs.

** 85. **Secretion of Saliva.**—The secretion of saliva goes on very slowly or ceases entirely when the glands are not under the influence of some stimulus. The stimulus may be either mechanical, chemical, electrical, or mental. The student may estimate the effect of different stimuli by experiments on himself, thus: Swallow all the saliva contained in the mouth, so as to empty it completely. At the end of two minutes spit out the saliva which has collected in the mouth into a small beaker previously counterpoised (*see* § 215) and weigh it. Again empty the mouth, apply the stimulus and collect the saliva for two minutes more, and weigh as before. By the comparison of the two, the action of the stimulus may be judged of. The best modes of stimulation are the following:—

1. *Mechanical.*—Roll a pebble or glass stopper in the mouth, and attempt to chew it.

2. *Chemical.*—Touch the tongue (1) with a crystal of tartaric or citric acid, or (2) of sodium carbonate; (3), fill the mouth with ether vapour, allowing it to pass back into the pharynx, and retaining it for some time in the mouth.

3. *Electrical.*—Touch the tongue, and inside of the cheeks with the electrodes of Du Bois Reymond's induction coil.

The effect which a stimulus applied to the mouth produces in man, on the secretion from the parotid and submaxillary glands, may be studied with greater precision by means of a cannula or syringe. If a syringe is used, its nozzle must end in a funnel-shaped dilatation. This is applied to the papilla at the orifice of Wharton's or Stenson's ducts, and gentle traction made upon the piston. A stimulus may be applied to the mouth, and the rate at which the saliva flows afterwards observed. It is, however, more satisfactory to use a cannula, which, with a little practice, can be introduced into the ducts with great ease.

* 86. **Mode of Collecting the Secretions of the Salivary Glands unmixed in Man.**—*Insertion of a Cannula into the Submaxillary Duct.*—Draw out a narrow glass tube to a fine point, and at the place where it seems small enough to enter the orifice of the duct, notch it with a triangular file, break it off, round the edges at the border of a gas flame and allow it to cool. To insert a cannula thus prepared into his own submaxillary duct, the student must now place himself before a mirror, with a bright light directed into the mouth. Fill the mouth with vapour of ether, or chew a piece of pyrethrum. Turn the end of the tongue back against the palate. At the root of the *frænum linguæ* a papilla with a little black dot is

seen at each side of the middle line. From these two dots, which mark the orifices of Wharton's ducts, the saliva will be seen to issue. Insert the end of the cannula into one of them, and hold it steadily in its place. The entrance of the cannula is attended with an unpleasant sensation, not amounting to pain. At first the cannula fills pretty rapidly, but as the effect of the ether passes off, the flow soon diminishes. If it is desired to collect the secretion, a piece of india-rubber tubing must be attached to the wider end of the cannula before inserting it.

Insertion of a Cannula into the Parotid Duct.—As it is hardly possible to insert a cannula into one's own parotid duct, a second person must be employed, who should sit opposite a good light and chew pyrethrum root as before. The method is as follows: Draw one angle of the mouth outwards and forwards so as to stretch the cheek. Opposite the second molar tooth of the upper jaw the small papilla is seen which marks the orifice of Stenson's duct. Insert the cannula and hold it steadily but carefully in its place, then a third person may blow into the mouth some vapour of ether, or introduce a little diluted tincture of pyrethrum.

By these methods a sufficient quantity of secretion can be collected for the investigation of the leading properties of the two secretions. Both possess the property of determining the transformation of starch and sugar.

87. **Study of the Secretions of the Salivary Glands in Rabbits.**—The ducts of the salivary glands in rabbits are too small to allow of the easy introduction of a cannula, but the secretion may be readily studied by cutting the duct across. The saliva escapes from the cut end and collects in drops. When the secretion is slight, it may be rendered readily visible by putting over the end of the duct a piece of bibulous paper reddened with litmus. The saliva is absorbed by the paper, and produces a blue spot, which increases in size, more or less rapidly according to the rate of secretion.

* *Parotid Gland.*—The duct runs from behind forwards across the masseter muscle about its middle, covered by fascia. It has branches of the facial nerve on each side of it, and is parallel with the transverse facial artery. At the anterior edge of the masseter it takes a direction towards the middle line, in order to enter the mouth.

If a vertical incision is made in a line with the cornea through the skin and fascia of the cheek down to the masseter, the facial nerves and transverse facial artery are cut across as well as the duct.

As soon as the bleeding has ceased, the discharge of saliva from the cut end may be investigated in the manner directed in § 90.

88. **Investigation of the Secretions of the Salivary Glands**

in the Dog.—**Permanent Salivary Fistulæ.**—Permanent fistulæ may be made either with or without inserting a cannula in the duct. In the method to be described, that of Schiff, no cannula is used. *Permanent Submaxillary Fistula.*—The animal having been placed on the table and its head secured with the aid of Bernard's holder, it is put under the influence of chloroform.* Shave the hair from the under surface of the lower jaw. Make an incision along the inner border of the ramus of the lower jaw, extending forwards from the anterior margin of the digastric muscle, and dividing the skin and platysma. Secure every vein that presents itself with two ligatures, and divide it between them. Divide the mylohyoid muscle cautiously. Underneath it will be found the submaxillary and sublingual ducts, which run side by side, the submaxillary being somewhat larger and nearer the ramus of the jaw. Isolate the duct and divide it as near as possible to its entrance into the mouth. Close the wound with sutures, leaving the end of the duct projecting. To prevent its retraction, pass a suture through it. When the wound heals, the end of the duct will come away, leaving a fistulous opening. Examine it daily, and if it has a tendency to close, pass a fine probe into it and along the duct.

Permanent Sublingual Fistula.—This is made in the same way as a submaxillary fistula, and the same animal may be used for both, but the two fistulæ should be on opposite sides of the head.

89. **Parotid Fistula.**—The animal having been secured and placed under chloroform as before, the hair is clipped from the cheek between the orbit and the angle of the mouth. On running the finger along the lower border of the zygomatic arch from behind forwards, its anterior and inferior root is felt at its insertion into the superior maxilla, forming an arch of which the convexity is directed backwards. At the end of this arch, between its insertion into the maxillary bone and the alveolus of the second molar tooth, a little depression is felt. Exactly on a level with this depression, and in a line with the insertion of the zygomatic arch, make an incision through the skin, cutting obliquely in a direction from the inner canthus of the eye towards the angle of the mouth. On dividing the subcutaneous cellular tissue, the facial vein and artery, a nerve, and the parotid duct will be found all together. The duct lies most deeply and runs from behind forwards, while the artery with its accompanying vein pass from above downwards. It is of a pearly white colour. Isolate it, and divide it as near the

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* In administering chloroform to a dog, great care must be taken that the vapour is sufficiently diluted with air, and that the sponge does not come into contact with the muzzle. The breathing must be carefully watched during the period of administration, and if it fails it must be continued by alternately compressing and relaxing the thorax. If this does not succeed, no time must be lost in opening the trachea and commencing artificial respiration.

mouth as possible. The wound must be closed round the duct, and the duct secured in it by a suture, just as in the case of the submaxillary gland.

* 90. **Effect of Stimuli on Secretion.**—In animals with permanent fistulæ, whether parotid or submaxillary, it can be demonstrated that these glands do not secrete excepting when secretion is excited by stimulants. The stimulation may consist in the introduction into the mouth of sapid substances, such as vinegar (which, in common with acid substances in general, acts most on the parotid), quinine, or colocynth, or of ether, or in electrical excitation of the tongue. The action of mental stimuli may be also shown, as, *e.g.*, by placing a bone before the nose of a fasting dog without allowing him to reach it. From Schiff's experiments, it appears that this kind of stimulation has no effect on either the parotid or submaxillary. The mastication of a bone produces an abundant secretion from both glands, but mastication of a tasteless substance, as, *e.g.*, a piece of wood, has no effect on the parotid, and a very slight one on the submaxillary. For rabbits a piece of hard biscuit should be used in place of a bone.

EXPERIMENTAL INVESTIGATION OF THE FUNCTIONS OF THE SUBMAXILLARY GLAND.

91. Owing to its comparatively exposed position, the submaxillary gland has been more completely studied than either of the other two. The investigation of its functions has yielded results which have acquired an importance far beyond that which they possess as bearing on the secretion of saliva. They form, indeed, the basis of all that is known as to the nature of glandular action, and of the influence exercised on it by the nervous system. Before proceeding to describe the methods employed, it will be necessary to give a short account of its anatomical relations, and particularly of the blood-vessels and nerves which are distributed to it.

Nerves.—The gland receives nerve fibres from three sources, viz., from the facial, from the submaxillary ganglion, and from the cervical sympathetic. The branch of the facial (known as the *chorda tympani*) reaching the neighbourhood of the duct, as part of the trunk of the lingual nerve, leaves that nerve as it crosses the duct, in order to accompany the latter to the gland (*see* fig. 307). In the angle which it thus forms with the lingual lies the submaxillary ganglion or ganglionic plexus above mentioned. From it fibres originate which reach the gland along with the chorda. The sympathetic fibres are derived from the superior cervical ganglion.

Physiologically, the nerves derived from the submaxillary ganglion cannot be distinguished from those of the chorda. When the chorda is irritated, the arteries of the gland dilate, the blood-stream becoming much more rapid; consequently, the veins leading from the organ pulsate, and if they are opened they jet like an artery. At the same time, the secretion discharged from the duct becomes copious and watery. When the sympathetic fibres are excited, the arteries contract, and the circulation in the gland is retarded, and if the veins are opened they discharge "black" blood in a slow stream. The secretion becomes scanty and tenacious.

It was first demonstrated experimentally by Ludwig that the increased secretion produced by excitation of the chorda is immediately dependent on increased activity of the function of the secreting elements of the gland, and not on changes in the blood-vessels; in other words, that in the submaxillary gland the process of secretion is not a mere filtration, but is effected by changes which go on within the gland itself, of such a nature as to determine a current from the circulating blood towards the duct. This conclusion was based by Ludwig on the observation: first, that if the duct is constricted, secretion continues, notwithstanding that the pressure in the interior of the gland is greater than that in the arteries; and, secondly, that secretion continues after circulation has ceased, *e.g.*, after the head has been severed from the body.

More recent observations make it probable that by the *chorda tympani* two kinds of fibres find their way to the gland, viz., fibres by which secretion is influenced directly, and others which are "vaso-inhibitory," *i.e.*, diminish arterial tonus. Among the most important observations bearing on this question are those lately published by Heidenhain, who has found that injection of atropia into the arteries or veins of an animal deprives the chorda of its power of over-secretion, without interfering with its vaso-inhibitory function; and the earlier experiments of Gianuzzi, made under Ludwig's directions, in which a similar effect was produced by the injection of solution of quinine, half-per-cent. hydrochloric acid, or five-per-cent. solution of sodium carbonate into the gland itself.

**** 92. Demonstration of the Functions of the Chorda Tympani and Sympathetic Fibres of the Submaxillary Gland in the Dog.**—The animal having been secured, as directed in § 88, and placed under chloroform, with the usual precautions, the hair is clipped from the jaws and neck, and the skin cleaned with a wet sponge. This having been accomplished, proceed according to the following

Directions.—1. Make an incision along the inner border of the lower jaw, beginning about its anterior third, a little in front of the insertion of the digastric muscle, and extend it backwards to the transverse process of the atlas, dividing the skin and platysma (*see* figs. 308 and 310).—2. Expose the jugular vein at or near the point where it divides into two branches (j′ and j″), and lay bare those branches also. One of them (j′) passes upwards behind the gland; the other (j″) passes forwards below it, and then subdivides into two branches. The gland itself has two veins. One of them (d′ fig. 308) issues from its posterior aspect and enters the vein j′. The other (d) comes from its lower side and enters the vein j″. Sometimes one vein (d) is larger, sometimes the other (d′).—3. Tie both branches of the lower division of the jugular opposite J″ (fig. 310). Tie the upper branch where it crosses the ramus of the jaw, and remove the part between the ligatures.—4. Tie the other division (J′) on the distal side of the place where it receives the vein (d′ fig. 308) from the gland.—5. Remove the cellular tissue from the surface of the digastric muscle, and from the groove between it and the masseter. Be careful not to injure the facial artery, and the duct of the gland which passes forwards and inwards between the muscles.—6. Separate the digastric muscle by means of a director or aneurism needle from the facial artery. Tie the arterial twig which supplies the muscle. Separate the muscle from its attachment to the jaw, or divide it about its anterior third, cutting it through very carefully, so as not to injure the duct and nerves which lie below it.—7. Lay hold of the lower end of the digastric with a pair of artery forceps, and draw it backwards. This brings into view a triangular space, whose apex is directed forwards, and whose base is formed by the reflected digastric. Its lower margin (the dog being supposed to be in the upright position, as in the figures) is formed by the genio-hyoid muscle, and its upper one by the ramus of the jaw and the lower edge of the masseter. The anterior half of its floor is formed by the mylo-hyoid muscle, on which some nerves ramify. The carotid artery enters the triangle at its lower angle, and runs along its base, giving off first the lingual artery, secondly the facial. Just as the carotid begins to pass in front of the digastric, it is crossed by the hypoglossal nerve P, and is accompanied by filaments of the sympathetic tt′. At the upper angle of the triangle, several structures pass from it to the hilus of the gland close to the margin of the digastric. These are—1, the duct; 2, the nerves; 3, the principal artery of the gland. The artery is given off by the facial at the upper angle of the triangle. It lies beneath the nerves, but is easily reached by drawing them aside. —8. Carefully isolate the digastric by a director or aneurism needle from all the structures just mentioned. Divide it close to its insertion into the temporal bone.—9. Divide the mylo-hyoid muscle, cutting its fibres across about their middle, and reflect the upper half, taking care not to injure the mylo-hyoid nerve which lies upon it, and tying all the veins which come into view on its surface with a double ligature. This brings into view the lingual nerve L, which issues from under the ramus of the jaw just opposite the groove between the masseter and digastric muscles, and after passing across the floor of the triangle towards the middle line, enters the mucous membrane of the mouth.—10. Draw the parts a little towards the middle line with the fingers, and follow the lingual nerve to the ramus of the jaw. A small twig T will then be seen, which passes off from its posterior aspect, bends down, making a sort of loop, and then runs backwards to the gland in close relation to the duct. This nerve is the *chorda tympani.* In the angle between the chorda and the lingual lies the submaxillary ganglion.—11. Isolate the *chorda tympani,* pass a thread under it, and tie the two ends together so that the nerve may be raised from its place at will.—12. Isolate the lingual nerve close to its entrance into the mouth, and pass a thread under it.—13. To reach the sympathetic, divide the hypoglossal nerve P just where it crosses the carotid, and raise up its central end. Close to the inside of the carotid lies the vagus, and when this is raised the sympathetic is seen underneath and inside of it. The sympathetic separates from the vagus at this point and goes to the superior cervical ganglion (*see* fig. 309). From the ganglion, fibres accompany the carotid and enter the gland, some along with the chief artery (O), and others with the other artery P′. The ganglion can easily be found by following the carotid filaments backwards.—14. Place a cannula in the submaxillary duct. The ducts of the submaxillary and sublingual glands pass along the middle of the triangle close to each other. The submaxillary duct lies nearer the ramus of the jaw, and is larger than the sublingual duct. Isolate it slightly with an aneurism needle. Pass under it a thread for the purpose of tying in the cannula. Place under the duct a smooth splinter of wood or a piece of card half an inch long by one-eighth of an inch wide, on which it may rest. Close the duct as near the mouth as possible with a clip, or tie a thread round it so as to obstruct it. Raise the chorda by the thread which has been passed round it, irritate it by a weak interrupted current: the purpose of this is to distend the duct with secretion, and thus render the introduction of a cannula much easier. Let an assistant lay hold of one edge of the duct with a pair of fine forceps while the operator lays hold of the other just over the splinter of wood on which it rests; open the duct between them with sharp-pointed scissors. Insert the cannula into the duct and tie it in.—15. Put a ligature round the jugular vein half an inch or an inch below its bifurcation, so as to be able readily to introduce into it a cannula when necessary.

In the preceding directions, all the steps of the operative procedure required for the complete investigation of the functions of he submaxillary gland during life are detailed. The method may, however, be modified, according as it is intended to limit the observation to the influence of direct or reflex excitation of the *chorda tympani* on the secretion of the gland, or to extend it to this investigation of the vascular changes and to the functions of the vascular nerves.

93. Direct and Reflex Excitation of the Chorda Tympani.—Proceed as above directed, omitting 13 and 15. 2, 3, and 4 may also be omitted, provided that all such veins as are necessarily involved in the succeeding steps are doubly ligatured and divided between the ligatures. *Reflex Excitation.*—Divide the lingual nerve close to its entrance into the mouth, and excite its central end with the secondary coil at a considerable distance from the primary. The secretion of saliva is increased. The animal must previously be allowed to recover from the chloroform, or no increase will be observed. The reflex action of the lingual is abolished during narcosis by opium, as well as by chloroform. ** *Direct Excitation.*—Divide the chorda close to the point at which it leaves the lingual, and place the peripheral cut end on the excitor (fig. 225), removing the secondary coil to a considerable distance from the primary. On opening the key, saliva is discharged from the cannula (to which an end of india-rubber leading into a test-tube has been fitted). It begins to flow a few seconds after the excitation, but *not* immediately. By repeating the excitation at regular short intervals, the discharge can be maintained, and a considerable quantity collected.

** **94. Demonstration that the Pressure produced by Secretion in the Duct of the Submaxillary Gland when it is Obstructed is greater than the Arterial Pressure.**—A cannula having been placed in the carotid of the opposite side of the body and connected with a mercurial manometer, a second manometer is connected with the cannula in the duct of the gland. The pressure indicated by the latter gradually increases until it attains a height greater than that indicated by the former. In this experiment it is desirable that the tube of the manometer connected with the duct should be narrow. Its proximal arm should be connected by a side opening with a pressure bottle at a height of about four feet from the table, the arrangement being the same as in the manometer of the kymograph. In this way a mercurial pressure of about 50 mill. of mercury is produced in the duct before excitation is commenced. On exciting the *chorda tympani,* it rises, as above stated, to double that height or more. For this experiment the same preparations are required as for the preceding, and the same animal may be used. The measurement of the arterial pressure in this experiment may be advantageously omitted. The pressure in the particular case may be assumed to be equal to the average.

** **95. Excitation of the Vascular Nerves.**—If the filaments which accompany the carotid or principal artery of the gland are excited, a few drops of secretion may be discharged, but the quantity is so small that unless care is taken that the cannula and duct are quite full before the key is opened, the effect will be scarcely perceptible. The secretion thus obtained is so thick and viscid, that the cannula is apt to become choked by it.

** 96. **Demonstration of the Influence of Excitation of the Chorda, and of the Vascular Filaments on the Circulation of the Submaxillary Gland.**—For this purpose it is necessary to insert a cannula into the jugular vein, which has been exposed for this purpose (*see* direction 15). In doing so, great care must be taken that the vein is not twisted, and that the cannula is properly inserted so as to allow the blood to flow freely out of it from the gland; it will be remembered that all the tributaries of the vein, excepting those from the gland, have been previously tied. On exciting the chorda, the blood flows from the cannula more rapidly, and acquires a brighter colour. The opposite effect is produced by exciting the vascular filaments.

97. **Simultaneous or Alternate Excitation of the Chorda Tympani and Vascular Filaments of the Submaxillary Gland.**—The same degree of excitation of the chorda which is sufficient to induce a marked increase of the secretion of the gland, is without effect if the sympathetic filaments are excited at the same time. Hence it is concluded that the functions of the two sets of fibres are antagonistic to each other, not only in relation to the circulation of the gland, but as regards their direct influence on secretion. The experimental proof of this consists in first exciting the chorda with the secondary coil at such a distance that the effect produced is only just appreciable, and then repeating the excitation while the vascular filaments are excited at the same time. In the latter case, the effect of the excitation of the chorda is annulled. If with a Pohl's commutator the same induced currents are directed alternately through the chorda and the sympathetic filaments at short intervals, the preventive influence of excitation of the latter manifests itself in the same way as if the excitation were simultaneous. Here, as before, the effect must be verified by comparative experiments.

98. **Simultaneous Section of the Chorda Tympani and Vascular Nerves.**—*Paralytic Secretion.*—After division of both nerves, the secretion of the submaxillary gland, which in the normal state only goes on when the gland is directly or reflexly excited, becomes constant and abundant. This effect does not occur until some time after section, and may last for days or weeks. A similar condition of the gland is produced by the introduction of curare into the blood, which is supplied to the gland by its arteries. To show this, proceed as follows:—Find the facial artery and prepare it. Then insert and secure a cannula, to which an end of india-rubber tubing has been previously fitted in the usual way. Fill

the cannula with saline solution, and connect it with the nozzle of a Pravaz's syringe previously charged with one-per-cent. solution of curare, taking care that the india-rubber tube is firmly tied round the nozzle. Open the clip, inject five divisions (about two milligr. of curare), and then close the clip. The same mode of injection may be used for the introduction of solution of atropin, if it is desired to repeat the experiments of Heidenhain previously referred to.

99. **Function of the Submaxillary Ganglion.**—Bernard found that excitation of the central end of the lingual, when divided near the mouth, produces effects similar to those of excitation of the chorda *i.e.*, causes the submaxillary gland to secrete even when the trunk of the lingual and chorda has been severed at a point nearer the brain than that at which it is in relation with the ganglion.

From this, Bernard concluded that the submaxillary ganglion acts as a reflex centre, independently of the central nervous system. More recent observations render it probable that Bernard's result derives its explanation from the anatomical fact that a filament of the chorda exists, at all events in some animals, which accompanies the lingual nerve for about an inch and a half beyond the point at which the chorda separates from it. The effect in question is to be attributed to excitation of this filament, which runs back parellel with the lingual nerve to the submaxillary plexus, and so to the gland. (On this subject, *see* Schiff, Physiol. de la Digestion, t. I., p. 288, and Haartman's Thesis, 1846. Helsingfors, p. 37, and Pl. I. 142.)

100. **Parotid Glands.**—In most animals the parotid, like the submaxillary gland, does not secrete unless the nerves which regulate its secretion are stimulated, but in the sheep it is said by Eckhard to secrete constantly. Secretion occurs when sapid substances are applied to the posterior part of the tongue, and still more when they are chewed; but the mere motion of the jaws in chewing a tasteless substance does not induce secretion. The gland receives two secreting nerves, one of which is derived from the facial, and the other from the sympathetic. The branch from the facial is the lesser superficial petrosal nerve, which leaves the facial in the petrous portion of the temporal bone, passes to the otic ganglion, and thence to join the auriculo-temporal branch of the fifth, in which it proceeds to the gland. These facts have been experimentally ascertained by observing, first, that irritation of the roots of the facial within the cranium determines flow of saliva from the parotid gland; secondly, that excitation of the fifth nerve within the cranium has no such effect; and, thirdly, that after section of the facial nerve at its exit from the stylo-mastoid foramen, the application of stimuli to the mouth determines secretion from the parotid as before. These facts, taken in combination, show that the secreting fibres for the parotid are

given off by the facial in its passage through the petrous part of the temporal bone. This conclusion receives direct confirmation from an experiment of Bernard, who found that destruction of the facial nerve in the temporal bone stops the secretion of the parotid.

Of the three nerves given off by the facial in its passage through the temporal bone, viz., the *chorda tympani*, the greater superficial petrosal and the lesser, the last-mentioned was proved by Bernard by exclusion to contain the secreting fibres for the parotid, for he showed that the chorda could be divided in the tympanum without affecting the parotid secretion; and as regards the greater superficial petrosal, it was known anatomically that it did not go to the parotid, and also found experimentally that excision of Meckel's ganglion had no effect on that gland. Bernard's conclusion has received direct confirmation from later experiments, which have shown, first, that the secreting function of the parotid gland is much impaired by the extirpation of the otic ganglion, and entirely annulled by section of the auriculo-temporal nerve. After division of this nerve, Schiff has shown that discharge of saliva cannot be induced by the application of stimuli to the mouth, and that electrical excitation of the peripheral end excites secretion just in the same way as excitation of the *chorda tympani*.*

** **101. Secretion of Saliva after Decapitation.**—Make a parotid fistula in a rabbit; decapitate it; split the head in the middle line by a knife and hammer; remove the brain from that half of the head on which the fistula has been made, apply a piece of filter-paper coloured red by litmus to the orifice of the duct, and irritate the roots of the facial as they enter the internal auditory foramen, either electrically or by touching the nerve with a drop of acid. A blue spot will appear on the paper, showing that saliva has been secreted.

SECTION II.—DIGESTION IN THE STOMACH.

102. In the stomach the albuminous constituents of the food which were unaffected by the saliva are dissolved by the gastric juice, and to a great extent converted into peptones. If they were merely dissolved, they could only be absorbed in very minute quantities, for albumin will hardly diffuse through animal membranes. The peptones into which the albuminous substances are converted, on

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* For a description of the method of dividing the facial at its exit from the stylomastoid foramen, see Eckhard's Beiträge zur Anatomie und Physiologie, Bd. III. p. 49. Section of the facial within the temporal bone is described in Bernard, Leçons sur la Physiol. et la Pathol. du Syst. Nerv., II. pp. 58 and 141. As regards section of the chorda in the tympanum, excision of the sphenopalatine ganglion, and division of the lesser superficial petrosal nerve, *see* Schiff, Physiol. de la Digestion, tom. I. p. 229. Excision of the otic ganglion, do. p. 227. For the method of exciting the auriculo-temporal nerve, *see* Nawrocki Stud. d. Physiol. Inst. zu Breslau, Ht. IV. p. 135.

the contrary, diffuse very readily, and are thus easily absorbed. The gelatinous substances in the food are also changed somewhat by the gastric juice, so that after they have been acted on by it they no longer gelatinize. The transformation of starch into sugar by the saliva, which was begun in the mouth, also goes on in the stomach, the acidity of the gastric juice being too slight to arrest it.

Unlike saliva, gastric juice cannot be readily obtained from man or animals, at any rate in a state of purity, without an operation. It is therefore necessary to establish a gastric fistula in a dog in order to collect a sufficient quantity of gastric juice for examination.

** 103. **Establishment of a Gastric Fistula.**—The object of making a gastric fistula is twofold: 1st, to obtain gastric juice for examination; and, 2nd, to observe the process of secretion within the stomach itself.

The method adopted by Bassow was simply to make an incision in the abdominal parietes, to sew the stomach to the edge of the wound, and then to make an opening in the stomach itself. The fistula was plugged with a piece of sponge. It was, however, very liable to close, and was too small to allow the interior of the stomach to be observed. Blondlot prevented the wound from closing by placing in it a cannula, which was closed with a cork, so that the gastric juice and products of digestion might not be lost during the intervals between his observations.

This method, as improved by Bernard, is the one usually employed. Bernard's cannula consists of two tubes, each of which has at one end a broad flange. One tube screws into the other, so that the distance between the two flanges can be altered at will. This is effected by means of a key which fits on two projecting points in the inner tube, and turns it round, while the outer one is held fast by the fingers. The advantage of this form over a simple tube with a shield at each end is, that the cicatrix of the wound often thickens in healing, and if the tube is not proportionately lengthened the outer plate presses on the skin and causes ulceration. The disadvantage of Bernard's cannula is, that it is too small to allow the interior of the stomach to be conveniently observed, and also, I think, that the edge of the wound comes into contact with the screw of the inner tube, and not with a smooth surface.

These disadvantages may be readily obviated by increasing the diameter of the tube and the width of the flange, and adapting a key to the projecting points by which the outer tube may be placed in the stomach and turned round as necessary. Such a cannula is represented in fig. 311.

104. **Operation for Gastric Fistula.**—Give the dog a hearty meal, so as to distend its stomach completely and make it lie

close against the intestinal walls.* Anæsthetize the animal by chloroform, taking care that the vapour is mixed with a sufficient proportion of air. Lay it on its back on the table, shave off the hair from the epigastric and hypochondriac regions, and remove the hairs carefully by a sponge, so as to prevent the risk of their getting into the peritoneal cavity. Make a vertical incision about an inch and a half to one side of the *linea alba,* preferably the left, and parallel to it, extending downwards from the lower edge of the costal cartilages to a distance somewhat less than the diameter of the flange of the cannula. Divide the muscles parallel to the course of their fibres. Tie every bleeding point before opening the peritoneum, so that no blood shall get into its cavity. Open the peritoneum on a director. Lay hold of the stomach with a pair of artery forceps at a point where there are not many vessels, and draw it forwards. Pass two threads with a curved needle into the gastric walls at a distance from each other about equal to the diameter of the tube of the cannula, and bring them out again at a similar distance from the points where they were introduced. Make an incision into the gastric walls, between the two threads, rather shorter than the diameter of the tube of the cannula. Put a pair of forceps, with the blades together, into the incision, and then dilate it by separating the blades till it is large enough to allow the cannula to be introduced. Push the cannula into the stomach up to its outer plate. Tie the stomach to it by the threads, and then pass their ends through the edges of the wound in the abdominal wall in such a way as to fasten the stomach to it, and at the same time to keep the cut edges in apposition. No other suture is required. Leave the cannula uncorked for at least half an hour after the operation is finished, for when the dog recovers from the chloroform it will vomit, and if the cannula be corked, the fluid contents of the stomach are apt to be forced past the side of the cannula into the abdominal cavity. Feed the dog on milk for one or two days, and if the operation be performed in winter, keep it in a place warmed night and day. The day after the operation the edges of the wound will be much swollen, but the swelling will subside in a day or two. After the wound has begun to heal, the cicatrix may thicken, and the outer plate of the cannula begin to press too much on the skin, so that it ulcerates. If this should occur, the cannula must be lengthened by screwing the two flanges farther apart. The cannula may be closed by an india-rubber stopper, or by a cork. If the dog tears out the cork with his teeth, soak it in decoction of colocynth, or put a little phosphoric acid on its outer end.

In order to collect the juice, let the animal fast for several hours, so that its stomach may be quite empty, but not for more than a day, as the mucous membrane would become covered with a thick coating of mucus. Let an assistant pat the dog, and keep him quiet; withdraw the cork from the cannula, and tickle the inside of the stomach with a feather tied to a glass rod. Put a small beaker underneath, so that the end of the rod rests on its bottom: the gastric juice will flow into it down the sides of the rod.

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* Holmgren recommends the inflation of the stomach with air, by means of a tube passed down the œsophagus, as preferable to distending it with food.

** 105. **Examination of Gastric Juice.**—The gastric juice is thin, almost colourless, very faintly opalescent, and has a faintly acid taste. Its specific gravity is nearly the same as that of water. Its reaction is strongly acid; blue litmus paper becoming bright red when dipped into it.

Composition.—In the dog, it contains three per cent. of solids; in man, only one per cent. About two-thirds of this is organic matter, consisting of pepsin and peptones; and one-third of inorganic matter, consisting of chlorides of potassium, sodium, ammonium, calcium, and phosphates of calcium, magnesium and iron. *The specific gravity and amount of solids*, organic and inorganic, are to be determined in the same way as those of saliva.

The acidity of the gastric juice is really due to free acid, and not to acid salts. To show this, the amount of bases and of acid contained in it must be determined. When this is done, it is found that the quantity of acid is more than sufficient to form acid salts with all the bases present which are capable of forming such salts; it must therefore exist partly in a free state. For the details of this process, consult Bidder and Schmidt, Verdauungssäfte, u. Stoffwechsel, 1852, p. 44; or Hoppe-Seyler's Handbuch d. Chemischen Analyse, third edition, p. 434.

106. **Estimation of the Acid in Gastric Juice.**—Fill a burette with dilute standard solution of soda (one part in ten), letting the standard solution flow gently into it, so as to avoid air-bubbles, till it is filled above the zero mark. Then place it in the stand, and take care that it is perfectly vertical. If any bubbles of air are present they must be allowed to break or be removed by a glass rod. Let the fluid flow out by pressing the clip till its level corresponds to the zero mark on the burette. Measure out 10 cubic centimeters of gastric juice into a beaker, and add a little litmus solution to it till a distinct red colour is produced. Place the beaker containing it under the burette, and let the alkaline solution flow gradually into it at first, and at last only drop by drop, stirring all the time till the red colour of the litmus changes to a violet. Then note exactly the level at which the surface of the fluid stands in the burette. The difference between this level and the zero mark gives the number of cubic centimeters used. Calculate the amount of soda contained in this quantity. One hundred

cubic centimeters of the original soda solution contained four grammes, or one-tenth of an equivalent of soda. One hundred cubic centimeters of the diluted solution therefore contains one-tenth of this amount, 0·04 grammes, or one-tenth of an equivalent.

Let us suppose that the amount of soda solution actually used to neutralize the gastric juice is 21·6 cubic centimeters. Then, as 100 cubic centimeters contain 0·04 grammes (=0·01 equivalent), this quantity will contain only 0·006 grammes (= 0·00216 equivalent). The quantity of gastric juice neutralized was 10 cubic centimeters. Had we used 100 cubic centimeters of juice instead of 10, we should have required ten times as much soda to neutralize it, *i.e.*, 0·0216 equivalent. One hundred cubic centimeters of the juice, therefore, contains 0·021 of an equivalent of acid, supposing that the acid be monobasic. If the acid be bibasic or tribasic, an equivalent of soda would only saturate a half or a third of an equivalent of acid, and the proportion of acid would be 0·015 or 0·007.

107. **To Determine the Nature of the Acid.**—The gastric juice is introduced into a large retort connected with a Liebig's condenser, and distilled till the fluid in the retort becomes very concentrated, and clouds begin to form in it. To remove the excess of water from the distillate, it must be neutralized with sodium carbonate, evaporated to dryness over a water-bath, extracted with absolute alcohol and filtered. The filtrate is then evaporated to dryness on a water-bath, and the residue dissolved in a small quantity of water. A little of the solution is now put in a test-tube, and a few drops of a neutral solution of ferric chloride added. If acetic acid is present, the fluid will become of a dark red colour, and when boiled will deposit a yellow precipitate. A solution of silver nitrate may be added to second portion. If hydrochloric acid is present, a white precipitate will fall, and will not be dissolved on adding nitric acid, but will be dissolved by ammonia. To the remainder, dilute sulphuric acid is added, and the mixture allowed to stand for some time. If butyric acid is present, a smell like rancid butter will be perceived. The residue of the gastric juice which remained in the retort after the hydrochloric and other acids were distilled off, is poured into a large test-tube or flask, and agitated with ether. The ethereal layer is then decanted off and evaporated over a water-bath. If acetic acid be present in the gastric juice, it will remain as an acid residue. Crystals of zinc lactate (square prisms with one or two oblique surfaces at the ends) may be obtained on allowing the residue to stand after the addition of zinc oxide and water.

108. **Action of Gastric Juice**—The power of gastric juice to dissolve coagulated albuminous substances is best shown by using

fibrin from blood. *To prepare fibrin* the blood is to be stirred as it flows from the vessel with a rough stick or piece of ragged whalebone, and the fibrin collected and washed till it is perfectly white. It may be preserved for a considerable time under glycerin, from which it must be washed before it is used. Put a small piece of fibrin into a test-tube along with gastric juice, and place the tube for an hour or two in the water-bath at 35° C. The fibrin will swell, become somewhat transparent, and then dissolve, forming an opalescent fluid, which is not precipitated by boiling, and slightly, or not at all by neutralization. As no other fluid except gastric juice has this action on fibrin, the production of all these effects is used as a test for it, and is called the pepsin test. Pepsin alone will not produce them, however, unless free acid be present as it is in gastric juice. In this process, boiled fibrin may also be used as recommended by Kühne.

** 109. **Artificial Gastric Juice.**—All the actions of gastric juice can be more conveniently studied with an artificial juice than with the natural secretion, as the former can be obtained in much larger quantities. The method of preparing it is as follows:—Open the stomach of a newly-killed pig or rabbit, or the fourth stomach of a calf, remove its contents and wash it thoroughly with a gentle stream of water without much rubbing. Lay it on a piece of board with its mucous surface upwards, fasten it down with a few pins, and then, with the back of a knife or an ivory paper-cutter, scrape off all the mucus from the surface. Rub it up in a mortar with clean silicious sand or powdered glass and water, let it stand some time, stirring it from time to time, and then filter it. The filtrate is gastric juice in a state of very considerable purity. It is slightly opalescent, and contains a large quantity of pepsin and but little peptone. When acidulated with its own bulk of dilute hydrochloric acid of 0·2 per cent., it digests fibrin with great rapidity. It may be kept in a bottle for a long time, and though fungi grow on its surface, it still retains its digestive powers.

A much stronger gastric juice, though not so pure, is obtained by scraping the mucus from the stomach as in the first process, or by dissecting off the whole mucous membrane from the muscular layer, cutting it into small pieces, then rubbing it up with dilute hydrochloric acid of 0·1 per cent. and filtering. The gastric juice so readily prepared by this method is very strong, and does very well for experiments on digestion, although it contains a good deal of albumin which is dissolved in the acid. It may be freed in a great measure from albumin by putting it into the water-bath at 35° C., for several hours, so as to convert the albumin into peptones, and then transferring it to a dialyzer, and changing the water several times.

The peptones will diffuse out into the water, a great part of the pepsin will remain in the dialyzer.

** 110. **To Prepare Hydrochloric Acid containing 0·2 per cent. of real H Cl.**—The ordinary strong hydrochloric acid sp. gr. 1·16 contains 31·8 per cent. by weight of H Cl. gas. To prepare a dilute acid, containing 0·2 per cent. of real H Cl., measure out with a graduated pipette 6·25 cubic centimeters of such acid into a litre flask; fill the flask up to the neck with distilled water, and shake so as to mix thoroughly.

** 111. **To Prepare a Solution of Pepsin in Glycerin.**—The solubility of digestive ferments in glycerin was discovered by Von Wittich; and by its means they may be obtained with great facility. Cut open the stomach of a pig or rabbit (best when newly killed), and wash the mucous membrane as directed; cut off the pyloric part; stretch the remainder on a piece of board, and dissect off the mucous membrane from the muscular layer. Cut up the mucous membrane into small pieces and put it into a beaker, with sufficient glycerin to cover it. It will acquire peptic properties in a few hours, but it is as well to let it remain for several days. Then strain off the glycerin and put on a fresh quantity. This may be repeated several times, and each time the glycerin will take up a fresh quantity of pepsin.

An artificial gastric juice may be readily prepared whenever it is wanted by adding a little of the glycerin extract to hydrochloric acid of 0·1 per cent.

** 112. **Preparation of Pure Pepsin from Glycerin Solution.**—Let the mucous membrane, prepared and cut into pieces, as already directed, lie for 24 hours in absolute alcohol. Filter off the alcohol; dry the pieces of mucous membrane with a cloth or filtering paper, cover them with glycerin, and let them stand for several days or weeks. Filter the glycerin, first through linen and then through paper. Add a large excess of absolute alcohol to the filtrate, and a flocculent precipitate will fall. Filter off the alcohol, pour H Cl. of 2 per cent. over the precipitate on the filter, and it will dissolve. Boil a little of the solution with strong nitric acid, and after cooling, add ammonia. It should not give the slightest trace of the xanthoprotein reaction. Let a piece of fibrin, either boiled or unboiled, remain in another portion of the solution for several hours, at 40° C., and it will be digested. Apply the other tests mentioned in § 118. Very probably no precipitate may be occasioned by platinum chloride.

113. **Preparation of Pepsin (Brücke's Method).**—The process by which Brücke separated pepsin, and thus for the first time succeeded in isolating any of the digestive ferments, depends on their being carried down from their solutions along with precipitates

produced in them. This has already been mentioned when speaking of saliva, from which Cohnheim separated ptyalin by Brücke's process. Separate the mucous membrane from the stomachs of two pigs, and cut it up into small pieces, as directed in § 109. Digest it at 40° C. with a considerable quantity of dilute phosphoric acid, of the British Pharmacopœia, mixed with its own bulk of water (it thus contains 5 per cent. of acid). If necessary, remove the acid, and add fresh portions till the whole of the stomach has been dissolved, with the exception of a slight residue, continuing the process till the liquid which passes through on filtering gives no precipitate with potassium ferrocyanide. Filter the liquid, put a little of the filtrate aside in a test-tube, and add lime water to the remainder till it turns blue litmus paper slightly violet. Collect the precipitate on a cloth filter, press all the fluid out of it with the aid of a screw-press, and dissolve it while still moist, in water, with the addition of dilute hydrochloric acid (50 cubic centimeters of commercial acid in a litre of water).

Precipitate the solution a second time with lime water, collect the precipitate on a cloth filter, press out the liquid, pour a little water on it while still moist, and add phosphoric acid to it in small quantities and at long intervals. The pasty tribasic phosphate $Ca_3 (PO_4)_2$ is thus converted into sandy bibasic phosphate $Ca\,H\,PO_4$. Filter off the fluid; it contains pepsin still mixed with albuminous substances. Test its digestive power by adding a few drops of it to 0·1 per cent. hydrochloric acid, and digesting fibrin in it. It will be found still to give the xanthoprotein reaction, though not quite so strongly as the original solution. Wash the precipitate upon the filter several times with distilled water, plug the funnel, and pour on dilute phosphoric acid, so that a part of the $Ca\,H\,PO_4$ is dissolved, $Ca\,H_4\,(PO_4)_2$, being formed. After several hours remove the plug, and let the fluid run off. It will digest fibrin, and has a still weaker xanthoprotein reaction. Wash the precipitate several times with distilled water, plug the funnel again, pour on fresh phosphoric acid, and repeat this several times. At last a fluid is obtained which, although it digests, gives scarcely any xanthoprotein reaction. *To prepare pure pepsin in substance,* prepare a solution with phosphoric acid and lime water, as directed above. After precipitating a second time with lime water, and pressing the precipitate, dissolve it in dilute hydrochloric acid and filter it into a large flask. Prepare a cold saturated solution of cholesterin in a mixture of 4 parts of alcohol of 808 sp. gr. and one part of ether. Put a long funnel which will reach to the bottom of the flask into it, and pour in the cholesterin solution in small quantities. It will separate and form a thick scum on the surface of the fluid. After it has attained the thickness of about an inch, take out the funnel, close the mouth of the flask and shake it well, so that as much pepsin as possible may stick to the cholesterin. Filter and wash the precipitate, first with water acidulated with acetic acid, and then with pure water. Continue the washing until the wash-water no longer has an acid reaction, nor gives a precipitate with silver nitrate. Put the moist cholesterin into a precipitate glass, and shake it with some ether which has been previously agitated with water to free it from alcohol. The ether will dissolve the cholesterin, and the adhering water will separate and form a turbid layer at the bottom of the glass. Pour off the ether and shake the watery solution with new quantities of ether several times, until a few drops of the ethereal solution no longer leaves behind crystals of cholesterin when evaporated. Then let the glass stand open, to allow the last thin layer of ether, which cannot be poured off, to evaporate. Filter; a small quantity of a slimy substance remains in the filter, but the filtrate is clear. It is a concentrated solution of pepsin, and the following reactions may be tried with it, or with the solution of pepsin obtained directly from the lime precipitate.

* 114. **Reactions of Pepsin.**—To show the following reactions the solutions referred to in §§ 112 or 113 may be employed. It is not precipitated by—1, concentrated nitric acid; 2, tannic acid; 3, iodine; 4, mercuric chloride. It is precipitated by—1, platinum chloride; 2, lead acetate, both neutral and basic.

If absolutely pure, it gives no xanthoprotein reaction. When allowed to evaporate over sulphuric acid, it leaves a greyish amorphous body, which contains nitrogen, and is not hygroscopic. It is sparingly soluble in water, more readily in dilute acids, and digests fibrin.

115. **Diges'ive Action of Pepsin.**—Neither pepsin alone nor dilute hydrochloric acid alone will digest fibrin, but when mixed together they do so readily. Pepsin alone has no action on fibrin whatever; hydrochloric acid of 0·2 per cent. alone causes it to swell up, but does not dissolve it for days, or even weeks, at ordinary temperatures. At 35°—38° C., it dissolves fibrin readily in from twenty-four to forty-eight hours, but only converts it into syntonin, so that the whole of the albuminous matter (with the exception of a trace which Von Wittich says is really converted into peptone), may be precipitated by neutralization. Pepsin with dilute hydrochloric acid likewise causes fibrin to swell and dissolves it, forming at first an opalescent solution of syntonin which can be almost entirely precipitated by neutralization, a little peptone only remaining in solution. Its action does not stop here, for it very quickly converts the syntonin (parapeptone) into peptones which are not precipitated by neutralization nor coagulated by boiling, but are precipitated by alcohol, and possess all the characteristic reactions of albuminous bodies.

116. Products of the Digestion of Albuminous Compounds. — During digestion several substances are formed, to which the names of parapeptone, dyspeptone, and metapeptone have been given by Meissner.

Parapeptone.—Brücke considers that albuminous bodies are converted into syntonin, and that the syntonin is transformed entirely into peptones during digestion, but Meissner thinks that the syntonin, instead of undergoing this transformation, splits up into peptones and parapeptones. Parapeptones agree with syntonin in every respect, except that they cannot be converted into peptones by any amount of digestion, while syntonin can be digested. When an albuminous body is subjected to the action of gastric juice, the solution first obtained yields, on neutralization, a precipitate of syntonin, which, when again treated with gastric juice, is converted into peptones. After digestion has gone on a little longer, the precipitate consists, according to Meissner, partly of syntonin and partly of parapeptones, for he states that if this precipitate is digested with fresh gastric juice, a less proportion of it than of the former precipitate is converted into peptones, and that this proportion diminishes more and more as digestion goes on, and the remaining syntonin is split up. Brücke and others have found, however, that fibrin can be completely converted into peptones; consequently, Meissner is not correct in supposing that syntonin splits up into peptones and parapeptones. Sometimes, however, several days are required to convert the whole into peptones.

Dyspeptone.—The dyspeptone of fibrin is a part of the syntonin or parapeptone, which becomes insoluble in 2 per cent. hydrochloric acid, and therefore falls as a fine precipitate. It also, according to Meissner, is incapable of further digestion, and only differs from parapeptone in being insoluble in dilute alkalies and dilute acids, and therefore is precipitated spontaneously from gastric juice without neutralization.

The dyspeptone of fibrin still requires investigation. The dyspeptone of casein has lately been examined by Hoppe-Seyler and Lubavin; as it consists partly, at least, of a non-albuminous substance, they consider casein to be composed, like hæmoglobin and vitellin, of an albuminous, combined with a non-albuminous, body.

Metapeptone is merely an intermediate stage between syntonin and peptone.

Peptones.—There are several kinds of peptones, but they still require further investigation. Meissner distinguishes three sorts, which he names *a*, *b*, and *c* peptones; *c* is the final product, the others being probably only preliminary stages in its production; *a* is precipitated from neutral solutions by concentrated nitric acid, and from solutions slightly acidulated with acetic acid by potassium ferrocyanide; *b* is not precipitated by concentrated nitric acid, but is precipitated by acetic acid and potassium ferrocyanide; *c* is not precipitated by either of these reagents.

** 117. **Demonstration of the Digestive Action of Pepsin.**—Take three test-tubes, and put into the first, water with a few drops of glycerin extract of pepsin; into the second, 0·1 per cent. hydrochloric acid; and into the third, the same acid with a few drops of the glycerin extract. Throw into each a small piece of fibrin, taking great care to choose pieces not only of the same size, but of the same texture, as hard pieces are much more slowly acted on either by acid or by gastric juice. Label each, or note the number of the hole in the rack in which each is placed, and put them all in the water-bath at 40° C. (fig. 331). In order to obtain a sufficient quantity of solution of peptones for testing, it is desirable at the same time to put a larger quantity of fibrin in a beaker with dilute acid, and when it has swollen up and become transparent, add some glycerin extract to it, and place it with the rest. Look at the test-tubes again in five minutes or so, and if the pepsin extract is strong, the bit of fibrin in the gastric juice will be partly dissolved, while the one in the acid will have swollen and become translucent, still retaining its form, while that in pepsin alone will be unchanged. Filter the artificial gastric juice from the residue of fibrin. Put a drop of litmus in the filtrate and neutralize it; a precipitate of syntonin or parapeptone will fall. Filter the liquid: the neutral filtrate containing peptones will not be precipitated by boiling, but it will give the xanthoprotein reaction strongly, and will give a precipitate with tannin.*

For the further examination of the products of digestion, filter the solution in the beaker from any undissolved residue. Neutralize, and parapeptones will be precipitated. Let the precipitate settle, and then filter: the filtrate will contain peptones. Test for *a* and *b* peptones. If they are present, put the beaker back in the bath for a while, and then test for them again. If they are no longer present, apply the following tests:—

** 118. **Reactions of Peptones.**—True or *c* peptones possess the following characteristics:—*They are not precipitated by* (1) neutralization, (2) boiling the solution, either neutral or acid, (3) nitric acid either in the cold or on boiling, (4) hydrochloric acid in the cold, (5) acetic acid and potassium ferrocyanide—(after standing, the fluid becomes turbid and gives a precipitate),—(6) copper sulphate in small quantity (if more is added it causes turbidity, which partly disappears on adding excess). They are precipitated by (1) tannic acid, (2) silver nitrate, (3) mercuric chloride, (4) platinum chloride, (5) lead acetate, both neutral and basic. (The precipitate is soluble in excess.)

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* For showing the action of pepsin to a class, Grünhagen's method may be employed. A piece of moist fibrin is placed in 0·2 per cent. hydrochloric acid till it swells to a stiff jelly. It is then laid on a funnel, either with or without a filter, and after the superfluous acid has drained off, a few drops of glycerin solution of pepsin, or artificial gastric juice, are added to it. The rapidity with which the fibrin is converted into peptone is shown by the number of drops which fall from the funnel. By using two similar filters, the power of different digestive fluids may be compared, and the effect of temperature shown by using Plantamour's funnel.

The solution when treated with caustic potash and an extremely minute quantity of copper sulphate, or a drop of diluted Fehling's solution, gives a precipitate which dissolves on shaking, and forms a *red* solution. If more copper sulphate is then added, it becomes violet. Peptones thus differ from albumin, which gives a violet at once.

** 119. **Diffusibility of Peptones.**—Put a solution of peptones into a small dialyzer, and let it diffuse into distilled water for an hour or two. Then test the water by the tests given above, and peptones will be found to be present. In this they differ from albumin, which, as has been already seen, hardly diffuses at all.

* 120. **Action of Gastric Juice on Gelatin.**—Pepsin, with dilute hydrochloric acid, deprives gelatin of its power to form a jelly sooner than dilute hydrochloric acid alone. Soak gelatin in cold water till it swells up completely, and then add sufficient boiling water to it to form a concentrated solution. Put some of it into two test-tubes, and add to each its own bulk of 0·2 per cent. hydrochloric acid. Put into one test-tube a little glycerin solution of pepsin, and into the other the same amount of glycerin, and place them in the water-bath at 40° C. Take them out after an hour or so, and let them cool. If both gelatinize, replace them for a while, and then cool them again, repeating the experiment if necessary. In this way the gelatin in the gastric juice will be found to lose its power of gelatinizing somewhat sooner than the other.

* 121. **Effect of Temperature on Digestion.**—A low temperature arrests the action of pepsin temporarily, but does not destroy its activity. It acts more and more rapidly as the temperature increases, until it attains its maximum between 30° C. and 50° C. Above this the action becomes slower. It is completely annulled by boiling. The activity of a dilute solution of pepsin is destroyed by exposure to a temperature of 70° C. for two minutes, and by a still lower temperature when exposed for a longer time. The activity of a concentrated solution is not so readily destroyed, and that of an undiluted glycerin solution is retained after being exposed to 80° C. for two minutes.

To show the action of temperature, take four tubes, and put into each equal quantities of 0·1 per cent. hydrochloric acid, to which a little glycerin solution of pepsin has been added. Put one in pounded ice, the second in a test-tube rack on the table, the third in the water-bath at 40° C., and boil the fourth, and then put it also in the water-bath. Put into each a bit of fibrin, and let them stand. The fibrin in the third tube will dissolve quickly, that in the second much more slowly, that in the first and fourth not at all. After a while—say half an hour—take the tube out of the ice and put it in the water-bath. The fibrin will then dissolve quickly, showing that the activity of the pepsin has been only suspended. That in the fourth will not dissolve at all, showing that the pepsin has been destroyed.

* 122. **Strength of Acid required for Digestion.**—The strength of acid with which albuminous bodies are most quickly digested by pepsin varies with the nature of the body, and also with the amount of pepsin present. Very dilute solutions of pepsin digest best with very dilute acids, while more concentrated pepsin solutions act more quickly with a somewhat stronger acid. There seems, indeed, to be a definite relation between the amount of pepsin and the strength of the acid, though what this is has not yet been determined. The proper strength of acid for any albuminous body may be ascertained by placing a number of test-glasses in pairs, the first pair containing very dilute acid, and each succeeding pair a stronger acid. In each glass is placed a little of the albuminous substance, and to one of each pair an equal quantity of solution of pepsin is to be added. They are then allowed to stand, and the rapidity with which digestion goes on in each is noted. The glasses with acid alone are required for the purpose of comparing its effects with those of the pepsin and acid together.

It can be shown as follows that digestion is hindered when the acid is either too weak or too strong:—Take three test-tubes, and put into the first 10 cubic centimeters of 0·1 per cent. hydrochloric acid, mixed with three times its bulk of water; into the second the same quantity of a similar acid undiluted; and into the third 9½ cubic centimeters of this acid, and half a cubic centimeter of commercial hydrochloric acid. Place in each a bit of fibrin, and put them all in the water-bath at 40° C. The fibrin in the second one will be quickly digested; that in the first and third tube much more slowly. The reason of the slow digestion in the third tube will be seen in the next experiment.

* 123. **Influence of the Swelling of Fibrin on its Digestion.**—If fibrin is prevented from swelling up under the action of gastric juice, either by mechanical means, such as a thread tied round it, or by chemical agents, such as salt solutions or too strong acids, its digestion is much retarded. Put about 10 cubic centimeters of 0·1 per cent. hydrochloric acid into four test-tubes, and add to that in the fourth test-tube half a cubic centimeter of commercial hydrochloric acid. Take four bits of fibrin as nearly as possible of equal size. Wind a thread firmly round one of them, and drop it into the third test-tube. Put another piece into each of the other tubes. As soon as that in the second tube begins to swell, add a saturated solution of sodium chloride to it till it shrivels again. Then add to the fluid in each tube half a cubic

centimeter of glycerin-pepsin, and let them stand. The fibrin in the first tube,which merely serves for comparison, is soon digested, and dissolves from without inwards. The bit in the second tube does not swell again, but dissolves from within outwards; so that a sort of shell remains, which, on shaking, falls to pieces. That in the third tube, which has been tied with a thread, behaves in the same way. That in the stronger acid, in the fourth tube, *swells incompletely,* but dissolves from without inwards, like the first.

** **124. Pepsin is not destroyed during Digestion.**—*Although the digestive power of pepsin appears to be indefinite, yet a limited quantity of gastric juice will not dissolve an unlimited quantity of fibrin.*—Add a little glycerin pepsin and a quantity of fibrin to some 0·2 per cent. hydrochloric acid in a test-tube, and place it in the water-bath at 40° C. for several hours. If all the fibrin is digested, add more, and repeat the addition until at last it remains undissolved, however long it may be digested.

The arrest of digestion in this experiment is not due to destruction of the pepsin, but to the accumulation of the products of digestion in the liquid, and to the want of acid.—Dilute the mixture with water, and put it in the water-bath again, and digestion will go on for a while and then stop. If again diluted, it will go on again, but the action will be slow from the dilution of the acid. If more acid be added, digestion will proceed more quickly, and by adding fresh quantities of acid, a very large quantity of fibrin may be digested.

The same thing may be shown by putting the fibrin and digestive fluid in a dialyzer and letting the peptones diffuse out. The digestive fluid is then to be evaporated to its original bulk, and acidulated, when it will digest the same amount of fibrin as it did at first. It is well to keep an excess of fibrin always in the dialyzer. This experiment is interesting, because digestion in the stomach takes place under somewhat similar conditions, the peptones being absorbed by the gastric vessels. *A stronger acid is required for digestion if the products of digestion are present in quantity in the solution.* When digestion stops, as in the previous experiment, it may be renewed by acidulating the solution more strongly with hydrochloric acid instead of diluting with water, and when it stops a second time a second addition of acid will set it on again. As too strong hydrochloric acid arrests digestion, a limit is soon put to the addition of acid, but if phosphoric acid is used instead, digestion may be kept up for a considerable time by fresh additions of acid.

* **125. Pepsin Test.**—The power of pepsin to dissolve albuminous substances and convert them into peptones, has been employed as a test for its presence. For this purpose either fibrin or coagulated white of egg may be used. The process is given by

Brücke in "Moleschotts Untersuchungen" for 1860, p. 490, and from this the following description has been taken:—

† *Pepsin Test with Fibrin.*—To test for the presence of pepsin in any substance or organ (as for example, any part of the digestive system of an invertebrate animal), it must be finely divided, treated with distilled water, and then allowed to stand for some time, with frequent stirring, and filtered. If the filtrate is alkaline it must be neutralized, after which as much hydrochloric acid must be added to it as will bring the percentage of acid to one-tenth. A bit of fibrin is then thrown into it; if it swells it is allowed to stand, but if it does not swell, dilute acid is added by drops at intervals till the edges and free fibres of the bits of fibrin become translucent. If the filtrate is acid, a bit of fibrin is thrown into it; if it swells up, it is allowed to stand, if not, acid is added as before directed till it does swell; the digestion is then allowed to go on at the temperature of the room, and the result observed.

The residue which remains on the filter, is introduced into a beaker covered with 0·1 per cent. hydrochloric acid, and placed in the water-bath at 40° C. for an hour and a half or two hours, or allowed to stand 24 hours at the temperature of the room, with frequent stirring. It is then filtered, and the filtrate used in the same manner as before. The reason why Brücke recommends that the watery extract should be tested separately from the acid extract, is that by this means pepsin already excreted from the peptic cells can be distinguished from pepsin still contained in them, inasmuch as the former is easily taken up by water alone, while the latter is taken up with difficulty by water, but easily by dilute acid. This process has also the advantage that when soluble albuminous bodies are present in any quantity, they are, for the most part, removed by the watery extract. If neither of these objects is of importance, the substance may be at once treated with dilute hydrochloric acid, and when it is small, as, for example, the salivary glands of insects, it may be at once thrown with a bit of fibrin into dilute hydrochloric acid, and digestion looked for. If a fluid is to be examined it must be filtered, and the filtrate and residue treated as above directed for solids.

Pepsin Test with White of Egg.—White of egg is more readily got than fibrin, but it dissolves more slowly, so that the test takes a longer time. Hard boiled white of egg, cut into dice, may be left for a long time in dilute hydrochloric acid without undergoing any changes, but the coagulum which is produced by boiling white of egg diluted with water undergoes partial solution pretty rapidly. The free alkali contained in white of egg is the cause of this difference in its behaviour when prepared in these different ways, and the inconstancy of its amount renders it difficult to determine what degree of acidity must be given to the liquid. To obviate this, add acetic acid to white of egg diluted with water until it turns blue litmus paper violet, but not red. Filter from the precipitate; test the reaction of the filtrate again, and correct it if necessary. Then coagulate it in the water-bath, wash it with water, and use it like fibrin, but use an acid of 0·15 per cent. If pepsin is present, digestion will go on just as with fibrin. The acid alone will not dissolve the albumin for many days.

126. **Theory of Pepsin Digestion.**—It has already been seen that neither pepsin alone, nor hydrochloric acid alone, will digest. C. Schmidt supposes that they do so when mixed, by forming a compound acid—pepto-hydrochloric acid. He thinks that digestion consists in the combination of this acid with albuminous bodies, and explains the fact that digestion can be renewed by the addition of hydrochloric acid after it has ceased, by supposing that the pepto-hydrochloric acid, thus liberated, is enabled to begin to digest anew.

The combination of pepsin and hydrochloric acid to form a new acid is supported by several facts, and is very generally believed, but Schmidt's hypothesis regarding its mode of action is open to the objection that it is not merely a compound of albumin with acid which is formed during digestion, but peptones. It therefore seems more probable that the pepsin acts as a ferment only in acid solutions, causing the albuminous bodies to take up water and split up.*

That pepsin and hydrochloric acid mutually combine when mixed, as in digestive liquids, is rendered probable, not only by the fact already shown that they produce effects together which neither is capable of producing separately, but that in such mixtures the characters of both are modified.

This is seen by comparing the action of dilute hydrochloric acid alone with that of hydrochloric acid pepsin. The former extracts all the salts and leaves a gelatinous substance, while the latter extracts this substance and leaves a brittle mass containing a large proportion of inorganic salts. As regards pepsin, a modification of property is shown in Von Wittich's observation, that although pepsin alone does not diffuse through vegetable parchment, pepsin with hydrochloric acid does so readily. That the decomposition of albuminous substances is essentially connected with their taking up water, is rendered probable by the fact that digestion does not take place in its absence, and that products similar to those of digestion can be obtained by boiling albminous bodies with water for a very long time, or for a shorter time with dilute acid.

The former of these facts can be easily demonstrated by treating fibrin which has been soaked in glycerin and not washed at 40° C. with a glycerin solution of pepsin undiluted with water, acidulated to the proper degree by the addition of a few drops of strong acid; under these circumstances the fibrin is not digested. The latter may be shown by boiling fibrin with dilute sulphuric acid for an hour or two, and then neutralizing the liquid, filtering and testing the filtrate for peptones.

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* For a clear account of the probable mode of action of ferments, *see* "Betrachtungen über die Wirkungsweise der ungeformten Fermente," by Dr. G. Hüfner; Barth, Leipzig, 1872.

* 127. **Secretion of Gastric Juice.**—Pepsin is contained in all parts of the peptic glands, but free acid is only formed near their orifices. To show this, kill a pigeon, open it immediately and dissect off part of the muscular layer from the proventriculus, which lies between the crop and gizzard. The ends of the gastric glands are thus laid bare. With a pair of curved scissors snip off the ends of the glands, taking care not to cut much below the surface. Squeeze the shred so obtained between two bits of blue litmus paper. It will have a neutral, or at most an extremely weak acid reaction, while the inside of the stomach will be found to be strongly acid. The presence of pepsin in the part of the glands where little or no acid is contained may be shown by dissecting off this part along with the muscular layer, and placing it in a test-tube with 0·1 per cent. hydrochloric acid in the water-bath at 40° C. Part at least of the muscular layer will be digested. The presence of acid only on the surface of the stomach can be shown, also, by injecting first a solution of half a gramme of ferric lactate, and then a solution of potassium ferrocyanide into the jugular vein of a rabbit, killing it about an hour afterwards, and opening the stomach immediately. These two salts form Prussian blue only in presence of an acid. On making a section of the wall of the stomach, it is seen that the blue colour is entirely confined to the surface, the deeper part of the mucous membrane remaining colourless.

After Death Acid continues to be formed in the Glands.—Thus, if the stomach of a pig or rabbit is cut in pieces, washed until it no longer gives a trace of acid reaction, and then left to itself, it is found after a time to be again acid.

* 128. **Digestion of the Stomach by itself.**—If there is only a small quantity of acid present in the stomach it will not be completely digested after death; but if it contains anything which will supply acid, not only the stomach, but a great part of the adjoining organs may be digested. Give a cat a quantity of milk, or introduce the same liquid into the stomach of a rabbit or guineapig by means of a syringe and a gum elastic catheter. Forth is purpose a perforated cork should be placed between the animal's teeth, and the catheter passed through the hole into the stomach. In an hour after kill the animal, and let it lie in a warm place for twenty-four hours. The whole of the stomach will probably be found digested. The stomach is not digested during life, because the alkalinity of its walls is preserved by the circulation of blood in them.

* 129. **Digestion of the Stomach during Life.**—When the circulation of the blood is arrested in one part of the organ, it becomes digested, and ulceration occurs. This is best shown by Sharpey's modification of Pavy's original experiment. The method consists in opening the stomach of a rabbit, narcotized by subcutaneous injection of chloral, by a longitudinal incision, seizing a part of its posterior wall with a pair of artery forceps and drawing it forward. This having been done, a ligature is passed round the part seized, so as to include a piece of about half an inch in diameter. Finally, the wound in the stomach and that in the abdominal wall are sewn up, and the animal placed in a warm place for some hours.

130. **Influence of Nerves upon the Secretion of the Stomach.**—The stomach, like the submaxillary gland, has two secretions; one thin, watery, and acid—the gastric juice proper; the other thick, tenacious, and alkaline—the gastric mucus. The latter is secreted and accumulates on the surface of the gastric mucous membrane during fasting, while the former is only secreted when an irritant is applied to the inside of the stomach. The irritant may be mechanical, *e.g.*, the friction caused by food, or any firm or hard substance introduced into the stomach, tickling with a feather, or rubbing with a glass rod. The most active chemical irritants are alkalies, which produce, even in very dilute solutions, an abundant secretion. This continues even after the alkali has been neutralized by the gastric juice or washed away by a stream of water. The saliva which is swallowed by the animal thus excites the secretion of gastric juice. Other stimulants are alcohol, ether, pepper, and cold water. When an irritant is applied, the gastric mucous membrane, which is of a pale colour, immediately becomes red; its vessels dilate like those of the submaxillary gland, and the watery-looking gastric juice oozes rapidly from its surface. The nerve centres, on which secretion is dependent, are present in the walls of the stomach itself, for it takes place even after all the nerves which enter the viscus from without have been divided. These centres are, however, as we shall see, much influenced by the vagi.

The *Action of the Vagus* on the stomach is still much disputed, but it would appear from the experiments of Bernard and Rutherford that it contains afferent fibres, the irritation of which, as, *e.g.*, during digestion, causes reflex dilatation of the gastric vessels. Bernard found that section of the vagi during digestion caused the stomach to become pale, and that in one or more experiments, irritation of these nerves reddened it, and induced an abundant secretion. He did not, however, determine whether this effect was due to afferent or efferent fibres, but Rutherford found that while section of the vagi during digestion, caused the stomach to become pale, irritation of their central ends generally reddened it. This effect was, however, sometimes preceded by its opposite, the organ becoming pale at first, and afterwards red, a result which indicates that the vagus contains two sets of afferent fibres, one of which increases, while the other diminishes the degree of contraction of the gastric vessels.*

** 131. **Effect of Stimuli on the Secretion of Gastric Juice.**—To see the effect of stimuli applied to the mucous membrane, a dog with a gastric fistula should be allowed to fast for six or seven hours, and then laid on its side in such a position that a good light falls into the cannula. The observation consists in noting the colour of the membrane, and then injecting a little dilute solution of sodium carbonate, or tickling the surface with a feather, and observing the effect. The effect of irritation on the amount of secretion may be estimated by letting the dog stand while a beaker is held under the cannula, and by measuring the juice which flows from it in a given time before and after irritation.

** 132. **Demonstration of the Action of the Vagus and Splanchnic on the Stomach.**—The proof that the vasomotor nerves of the stomach are derived from the splanchnics is founded on the observation that when the left splanchnic is irritated in the rabbit, as directed at page 249, the arteries at the great curvature may be seen to contract. This may be still better seen in the cat.

The vagus is the sensory nerve of the stomach, and contains afferent fibres, the irritation of which produces reddening of the gastric mucous membrane. It also contains motor-fibres which are distributed to the muscular coats of the organ. To show these facts, a cat must be placed under chloroform, after which both vagi are prepared, and the stomach exposed. If now, the animal having partially recovered from the anæsthetic, the stomach is seized between the thumb and forefinger, and subjected to traction in the direction of its length, slight but unequivocal signs of uneasiness are perceived. The vagi are then divided, after which it may be observed, first, that the stomach is paler than before, and, secondly, no sign of uneasiness is produced by traction.

On irritation of the central end of one of the divided nerves, the colour of the mucous surface is more or less completely restored. On irritation of the peripheral end, the walls of the stomach often begin to contract, but this effect is not constant when either splanchnic is intact. When both are divided, irritation of either vagus is invariably followed by movements of the stomach (Houckgeest).

Experiments on vomiting have been omitted, as they do not succeed in narcotized animals.

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* From the observation of Bernard and Blondlot, that gentle excitation increases the secretion of gastric juice while violent irritation stops it and causes vomiting, it appears probable that some of the gastric nerves are more easily excited than others. *See* Carpenter's Physiology, edited by Power, 7th edition, p. 128.

SECTION III.—FUNCTIONS OF THE LIVER.

BILE.

133. **General Characters of the Bile.**—Bile as it flows from the liver is a thin liquid, but when it stays some time in the gall bladder it becomes mixed with mucin, the presence of which renders it tenacious. In man, it is, when fresh, of a golden-yellow colour, like yolk of egg, as may be seen when it is vomited; but after death the bile in the gall bladder is generally brownish. In the dog it is also yellow, in the herbivora it is green, but very frequently it has a decided brown tinge in both. Its specific gravity and composition are not always the same even in the same animal.

Specific Gravity and Solids.—The specific gravity and amount of solids, organic and inorganic, in bile are determined in the same way as in saliva. The ash has a reddish tinge, due to the presence of iron. For the method of determining the amount of iron, *see* page 193.

* *Reaction.*—Bile discolours litmus so much as to hide the reaction, it must therefore be first diluted and the reaction tested afterwards. In fresh bile it is always alkaline.

134. **Composition of Bile.**—When obtained from the gall bladder, the bile contains 1, mucin; 2, bile pigments; 3, sodium salts of biliary acids; 4, cholesterin; 5, lecithin; 6, phosphates of sodium, calcium, and iron, sodium chloride, and generally traces of copper.

* *Mucin.*—Add common alcohol to bile, obtained from the gall bladder of an ox; wash the abundant precipitate so obtained with dilute alcohol; add water, and the precipitate will dissolve; add acetic acid, and a precipitate of mucin will fall with traces of bile pigment adhering to it. For the reactions of mucin, *see* § 45.

Bile Pigments.—The yellow colour of fresh bile in man and carnivora is due to a colouring matter termed *Bilirubin;* the green colour possessed by the bile in herbivora, or acquired by the bile of carnivora after standing, is due to *Biliverdin,* a product of the oxidation of Bilirubin. When the bile is long in the gall bladder, a small quantity of a third pigment, *Biliprasin,* may also be present.

** 135. **Test for Bile Pigments. (Gmelin's Test.)**—When strong nitric acid, which has been exposed to light, and therefore contains nitrous acid, is added to a solution of bilirubin, it becomes oxidized, and the products of oxidation which are successively produced, present the colours of the rainbow. First, biliverdin is produced, and the yellow colour of the bilirubin solution changes to green, and then becomes successively blue, violet, red, and lastly dirty yellow. If a solution of biliverdin is used instead of bilirubin, the same changes of colour occur, but the first change is of course to blue. In the reaction above described, the oxidation is most complete at the point of contact of the two liquids, the degree of action diminishing as the distance from this point increases. If, therefore, the two liquids are brought into contact without agitation, successive zones of colour are formed by the products of oxidation in the same order as before, viz., green, blue, violet, red, and dirty yellow, the last mentioned being nearest the acid. In order to apply the test to a fluid supposed to contain bile pigment, pour it in a thin layer on a white porcelain plate, and place two or three drops of nitric acid in contact with its edge. Or pour nitric acid containing a *little* nitrous acid into a test-tube: hold it obliquely, and let the fluid to be tested flow gently down the side of the test-tube and over the surface of the acid. Fix the test-tube in the same oblique position, without shaking, in a holder, and let it stand; see from time to time whether the rainbow-coloured zones have appeared at the point of junction in the proper order. *Brücke's method* is to mix the fluid to be tested with very dilute nitric acid, and then to let a little strong sulphuric acid run gently down the side of the test-tube. Dilute nitric acid alone does not act on the bile pigment, but after the addition of the sulphuric acid the coloured rings spread from its upper surface. Ox gall does not exhibit the coloured zones, even when treated with strong nitric acid, unless it contains much nitrous acid.

To show them, pour a little ox-bile on one part of a porcelain plate, and on another near it some very strong nitric acid, containing much nitrous acid, or nitric acid, previously mixed with concentrated sulphuric acid, and let the bile and acid gently come in contact.*

Fallacies to be avoided in using Gmelin's Test.—This test must never be applied to a fluid containing alcohol, as the alcohol alone will cause abundant formation of nitrous acid, and produce the coloured rings, although no bile pigment is present.

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* When the urine of a patient suffering from jaundice is tested for bile pigments with nitric acid, the colour reaction sometimes cannot be obtained, even though the urine be so dark that the foam on its surface, after shaking it, is quite yellow. This negative result generally occurs in cases where the temperature of the patient is high, and more especially when it has continued high for some time. It is then advisable, instead of testing the urine directly with nitric acid, to use the method recommended by Huppert. Precipitate the urine with milk of lime, throw the precipitate on a fluted filter, and allow the fluid to drain away. Take a piece of the precipitate, about the size of half a hazel-nut, place it in a test-tube, fill the tube half full of alcohol, and then add dilute sulphuric acid in such quantity that the fluid, after being shaken, has an acid reaction. Warm the tube: the fluid will extract the colour from the precipitate; filter and boil the filtrate. If bilirubin is present in the urine, it will combine with the lime and be precipitated; but it will be again set free by the sulphuric acid, and be dissolved by the warm acidulated alcohol, forming a yellowish-green solution. This solution will become dark green on boiling, and the more free acid present, the sooner will it do so. When long boiled, it sometimes becomes blue.

In using it for the detection of bile pigment in urine, the presence of indican may lead to error. This is avoided if care is taken to observe that the green, violet, and red zones are all present, as urine containing much indican may exhibit green and yellow zones alone, or green and yellow with blue between, but never exhibit all of the colours in the right order.

* 136. **Bilirubin.** $C_{16} H_{18} N_2 O_3$.—Synonymes: Bilifulvin, Biliphain, Cholepyrrhin, Hæmatoidin.

Preparation from Bile.—Put some fresh dog's bile in a small flask, acidulate it with acetic acid, add chloroform till the flask is almost full, warm it in a water-bath, and shake. The chloroform takes up the bilirubin and settles at the bottom of the flask. Remove it with a pipette, and evaporate it quickly. The red residue is bilirubin. Add alcohol to it to dissolve out the impurities; pour it off after it has stood some time; dissolve the bilirubin again in chloroform, and again evaporate. To obtain it pure, this may be repeated once or twice. When crystallized, it is of a red colour. During crystallization, a part of it is apt to become oxidized with biliverdin, on which account it is easier to obtain it pure by precipitating it from the chloroform solution by the addition of alcohol. The precipitate is amorphous, and of an orange colour.

The amount of bilirubin which can be obtained from the bile of a single dog is very small. To obtain it in greater quantities, biliary calculi may be used.

Preparation of Bilirubin from Gall Stones.—Reduce the gall stone to powder, and extract it first with ether, to free it from fat and cholesterin, so long as any of the powder is dissolved; next boil it with water, to free it from admixture of bile; and lastly, treat it with dilute hydrochloric acid, to remove lime and magnesia. Dissolve the residue in warm chloroform. Filter (preserving the undissolved part), distil, or evaporate off the chloroform; extract the residue with absolute alcohol (preserving the alcoholic extract, *see* § 147), and then with ether. Dissolve the residue in a second quantity of chloroform, and evaporate until the bilirubin begins to separate, and then precipitate it with alcohol.

Properties of Bilirubin.—The orange-coloured precipitate is, 1, quite insoluble in water; 2, very slightly soluble in ether; 3, sparingly soluble in alcohol, but rather more soluble than in ether; 4, soluble in chloroform, especially when warm, and in a less degree in benzol and bisulphide of carbon, amyl-alcohol, and glycerin. Its solutions have a yellow or brownish-red colour, which is so intense that it is distinguishable in a layer 1·5 centimeters thick of a solution containing one part in 500,000.

Bilirubin combines with alkalies, forming compounds which are soluble in weak alkaline liquids, which are precipitated by neutralization. They are insoluble in chloroform, the chloroform solution bilirubin being precipitated by alkalies. For this reason it is necessary to acidify bile before extracting the bilirubin with chloroform. *Bilirubin also combines with Lime.*—If bilirubin is dissolved in ammonia, and the solution precipitated with calcium chloride, a rusty-red flocculent precipitate is obtained, which is a calcium compound of bilirubin.

* 137. **Biliverdin.** $C_{16} H_{20} N_2 O_5$ or $C_{16} H_{18} N_2 O_4$—*Preparation.*—Put an alkaline solution of bilirubin in a flat shallow vessel, and let it stand exposed to the air for a considerable time, until it becomes green. Precipitate it with hydrochloric acid, wash the precipitate with water, dissolve it in alcohol, filter, and evaporate. The biliverdin is left as an amorphous body. The reaction by which bilirubin is converted into biliverdin is considered by Staedeler to be $C_{16} H_{18} N_2 O_3 + H_2 O + O = C_{16} H_{20} N_2 O_5$.

Properties.—It is insoluble in water, ether, and chloroform. It is soluble in 1, alcohol (and can thus be separated from bilirubin, which is insoluble), 2, dilute liquor potassæ, or 3, ammonia, and 4, strong sulphuric acid. It is precipitated from its alkaline solution by acids, or by salts of calcium, barium, or lead. It is precipitated unchanged from its solution in sulphuric acid by the addition of water. Nitric acid oxidizes biliverdin in alkaline solutions, and produces the same series of colours as with bilirubin. Sulphurous acid, which is a powerful deoxidizing agent, causes alkaline solutions of biliverdin, especially when warmed, to become yellow; when the yellow solution is treated with nitric acid, it behaves just like a solution of bilirubin.

138 **Relation of Bile Pigments to Hæmoglobin.**—Bilirubin is generally believed to be formed from hæmoglobin, which becomes altered during the passage of blood through the liver. The grounds for this belief are the apparent identity of bilirubin, and the pigment called hæmatoidin, found in old extravasations of blood, and the observation that bile pigments appear in the urine after the injection into the veins of solutions of hæmoglobin or of any substance which will dissolve the blood corpuscles, and liberate hæmoglobin, such as water (Herrmann), bile acids (Frerichs Kühne), or ether (Tiegel). They also appear after prolonged inhalation of ether (Nothnagel), or chloroform (Bernstein). Further support is also lent to this view by the destruction of hæmoglobin, which appears to take place in the blood during its passage through the liver (Gréhant). Though a positive result has been obtained by so many observers, Naunyn failed to detect bile pigments in the urine of rabbits after the injection of hæmoglobin, either subcutaneously or into the jugular vein, and attributed the

success of others to their experiments having been made on dogs, in whose urine bile pigment is normally of frequent occurrence. He noticed them, however, in rabbit's urine, when blood in which the corpuscles had been destroyed by freezing or ether, was injected into the intestine, so that the hæmoglobin absorbed from it, or set free by the action of the ether on the blood of the portal vein, passed through the liver before reaching the general circulation. Naunyn's experiments, also, have been repeated by Wolff and Wickham Legg, with a negative result.

In performing them proceed as follows:—Narcotize a rabbit with chloroform, shave the hair from the belly, make an incision about 1½ centimeters in length in the linea alba a little above the middle point, between the base of the xiphoid cartilage and the symphysis pubis. Seize a coil of small intestine with a pair of artery forceps, and hold it opposite the wound, without drawing it forward more than is just necessary. Inject 2 cub. cent. of ether into the intestine close to the points of the forceps with a subcutaneous syringe. Tie a ligature round the point wounded by the syringe and forceps; attach the intestine by it to the abdominal wall, and close the wound with a point of suture. The inhalation of chloroform is too short to produce of itself bile pigment in the urine, and it greatly facilitates the operation.* Examine the urine of the rabbit for bile pigments an hour or two after the operation, and again next morning. To get the urine, hold the rabbit over a large beaker, compress the abdomen with the palm of one hand, and press with the thumb of the other on the bladder just above the pubes, pushing it well down into the pelvis.

139. **Relation between the Colouring Matter of Bile and that of Urine.**—The urinary pigment is supposed to be derived from that of bile, as a substance which presents similar spectroscopic characters can be extracted from bile, or produced by deoxidation from bilirubin. In the organism, bile pigments are probably reduced by hydrogen, or other reducing agents present in the intestine.

When dog's bile is extracted with dilute hydrochloric acid and filtered, the filtrate has a reddish or reddish-yellow colour, and on spectroscopic examination presents a band close to F, between it and *b*, which disappears on the addition of liquor sodæ, and is replaced by a narrower band, also between *b* and F, but nearer *b*, the filtrate at the same time assuming a yellowish colour. If the solution is only very slightly alkaline, both bands may be seen at once. Ammonia produces similar changes in the colour of the fluid, but the second band is very faint when it is employed.

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* The writer has failed to observe bile pigments in the urine, either after the injection of bile acids into the veins, or of ether or dissolved blood corpuscles into the intestines.

On acidulation, the alkaline liquid regains its red colour, and the first band re-appears. By treating it with chloroform, a solution is obtained in which the first band is visible, but is somewhat nearer *b*.

Urine, especially when high-coloured, exhibits the band at F, though not very distinctly; but it may be clearly seen by precipitating the urine with lead acetate, decomposing the precipitate by an acid, and examining the filtrate spectroscopically. The addition of sodium hydrate causes the other band faintly to appear, and when treated with chloroform in the same way as bile, the solution and the position of the band seen in the chloroform solution is altered in a similar manner.

A substance presenting a similar band is obtained by acting on a solution of bilirubin in liquor potassæ or liquor sodæ with sodium amalgam, for several days, with exclusion of air (Maly).

** 140. **Bile Acids.**—The bile acids are taurocholic and glycocholic acids. In the bile of the pig another acid, hyocholic acid, is present. In the bile they are combined with soda, and their soda salts form the so-called crystallized bile. These acids are conjugate acids, composed of cholic acid in combination with taurine and glycocine. The presence of cholic acid or its compound is recognized by a reaction known as

Pettenkofer's Test.—This test shows the presence only of bile acids, but not of bile pigments or other constituents of bile. Dilute some ox bile with water and filter it. Put a little in a test-tube, with a small piece of sugar or a little strong syrup. Then add concentrated sulphuric acid drop by drop, shaking the tube after each addition; the temperature of its contents should be kept as near 70° C. as possible, either by warming it if necessary, or putting it in cold water if it gets too hot. Cholic acid is first precipitated and then dissolved by the sulphuric acid, the solution assuming a cherry-red and then a beautiful purple colour, which becomes gradually darker when the liquid is allowed to stand. The reaction is hindered by the presence of much pigment, oxidizing substances, and albuminous bodies, or bodies readily decomposed by sulphuric acid. It is therefore better to use a solution of crystallized bile, if it is at hand, than diluted bile. This reaction cannot be relied on alone as positive proof of the presence of bile acids, for amylic alcohol and other organic substances give a similar coloration. To show this, put a solution of albumin, or rather of syntonin (§ 7), into a test-tube with a little syrup, and add strong sulphuric acid. A purple colour is developed. To distinguish between the purples given by bile and by albumin, examine the test-tubes by the spectroscope. The bile acids give four bands; the first at D, the second and third between D and E (the second being nearer D, the third close to E), the fourth at F. If the solution is dilute, the third band is seen sharply, the second less distinctly, and the other indistinctly. The coloured albuminous solution gives only one band between E and F.

Detection of Bile Acids in the Urine.—They are usually present only in small quantities in the urine, even in severe cases of jaundice. Various methods of applying Pettenkofer's test have been proposed, one of which (Strassburg's) is applied as follows :—Add a little cane sugar to some urine containing bile acids, dip a piece of filtering paper into it, let it dry completely, put a drop of pure sulphuric acid upon it, and allow the acid partially to run off. In a quarter of a minute a beautiful violet colour appears, which is best seen by holding up the paper to the light and looking through it. In all doubtful cases, and whenever accurate results are required, the bile acids should be separated before applying the test, *see* § 204.

* 141. **Crystallized Bile.**—*Mode of Preparation.*—Evaporate bile to a quarter of its volume, mix it with a considerable quantity of animal charcoal, rub them thoroughly together, and then heat the mixture on a water-bath till it is *perfectly* dry. Put it immediately, while still warm, into a flask, cover it with absolute alcohol, cork the flask, and let it stand for a good while, shaking it occasionally so that the alcohol may dissolve out all the bile salts. Filter, and pour the filtrate into a perfectly dry stoppered bottle, large enough to hold four times as much. Add ether to it, until no further precipitate is produced; then replace the stopper, and put the bottle aside for a few days. If the alcohol and ether are both anhydrous, the precipitate which falls consists of microscopic crystals, but generally it forms a resinous mass at the bottom of the flask, which after several days, or weeks, begins to crystallize, and groups of silky needles appear.

To preserve the crystals, pour off the mixture of alcohol and ether, wash them with pure ether, evaporate the adhering ether from them *in vacuo,* and replace the stopper in the bottle. The crystals, if left exposed, take up moisture, and form a resinous mass, which is eventually converted into a syrupy fluid. Crystallized bile is very soluble in water and in alcohol, but insoluble in ether.

Composition of Crystallized Bile.—Crystallized bile consists of the sodium salts of glycocholic and taurocholic acids. To separate these two acids from the base and from each other, dissolve the crystals or the resinous precipitate in water, and add first solution of neutral lead acetate, and then a little basic lead acetate. This combines with the glycocholic acid, and forms an insoluble lead-glycocholate. Filter, and add to the filtrate lead acetate and ammonia, and a precipitate of lead-taurocholate will be formed. Filter; the filtrate contains the soda which has been set free, and also the excess of lead. The nature of the base may be shown by precipitating the lead from the solution by hydrogen-sulphide, and filtering; the filtrate when evaporated to dryness leaves sodium acetate.

* 142. **Glycocholic Acid** ($C_{26} H_{45} NO_6$) is abundant in ox gall, but is only present in small quantities in human bile, and absent from the bile of the dog and cat. *Preparation.*—Dissolve the lead-glycocholate obtained in last experiment in hot alcohol; precipitate the lead with hydrogen-sulphide, concentrate the alcoholic solution by evaporation, and then precipitate the glycocholic acid by adding water.

Another and easier plan is that of Gorup-Besanez. Evaporate ox gall nearly to dryness in a water-bath, and exhaust the residue with alcohol of ninety per cent. (sp. gr. 822). Distil or evaporate off the alcohol, dilute the residue if necessary with water, add milk of lime to it and warm it gently. The greater part of the colouring matter will be precipitated by the lime. Filter, allow it to cool, and add dilute sulphuric acid to it (avoiding excess), until a permanent turbidity is produced. Let it stand for a few hours, and the fluid will in most cases become a mass of crystals of glycocholic acid. Occasionally this conversion does not take place till after some days, or even weeks. Throw the mass on a filter connected with the water air-pump, wash with cold water, and press it between folds of blotting paper, first with the hand and then with a screw-press. It may be obtained in a still purer condition by dissolving it in a large quantity of lime-water, and adding dilute sulphuric acid until the glycocholic acid again separates. It crystallizes in long thin white needles. The crystals are sparingly soluble in cold water, more readily in warm, from which it crystallizes out on cooling. It is very sparingly soluble in ether, readily in alcohol. When water is added to the alcoholic solution, the acid is precipitated first as a turbidity, and then in flakes and drops, which become gradually converted into crystals.

* 143. **Glycocine or Glycocol.**—Glycocholic acid can be decomposed, and glycocine obtained from it by boiling it for a long time with strong hydrochloric acid.* On then allowing it to cool, a

resinous mass (cholalic acid and dyslysin) separates. The fluid is poured off from the resin and evaporated. The residue is then dissolved in water warmed with hydrated lead oxide, and filtered; the filtrate decomposed by hydrogen-sulphide, filtered, and the filtrate evaporated.

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* Glycocine is more readily prepared from hippuric acid, which is contained in large quantities in the urine of herbivora, and consists of glycocine in combination with benzoic acid. *Preparation of Hippuric Acid.*—Milk of lime is added to horse's or cow's urine; the mixture is boiled, filtered, neutralized with hydrochloric acid, and evaporated to a small bulk. On acidulating with hydrochloric acid, hippuric acid crystallizes out in rhombic prisms resembling thick needles (fig. 313). *Glycocine* is prepared by boiling hippuric acid with strong hydrochloric acid for several hours, and evaporating the solution almost to dryness. The hippuric acid is decomposed, yielding benzoic acid and glycocine. The residue is extracted with cold water, which dissolves but little of the benzoic acid. To the watery extract hydrated lead oxide is then added, to remove the hydrochloric acid. The liquid is filtered, and the lead precipitated from the filtrate by hydrogen sulphide. The precipitate having been removed by filtration, the filtrate is evaporated to a small bulk.

The transparent rhomboidal crystals of glycocine thus obtained are then washed with absolute alcohol. They have a sweet taste, and are readily soluble in cold water; almost insoluble in ether and alcohol.

* 144. **Taurocholic Acid** ($C_{26}H_{45}NSO_7$) is present along with glycocholic acid in ox bile; it is the chief acid in human bile, and the only one in that of dog's. *Preparation.*—Suspend the lead-taurocholate obtained from crystallized bile in alcohol, and decompose it by hydrogen-sulphide: filter; evaporate the filtrate at a moderate temperature to a small bulk, place it in a stoppered bottle, and precipitate by a great excess of ether. The acid is precipitated as a syrup. After standing, it changes, if the process is successful, to fine silky crystals, which, when exposed to air, dissolve, or form a syrup.

Taurocholic acid is soluble in water and alcohol, insoluble in ether. It is recognized as a bile acid by giving Pettenkofer's reaction, and is distinguished from glycocholic acid by not being precipitated by lead acetate alone, but by lead acetate and ammonia, and from any other bile acid by yielding taurin when decomposed by boiling with hydrochloric acid. It may be prepared from taurocholic acid or from crude bile.

145. **Taurine.** ($C_2H_7NSO_3$)—*Preparation.*—Boil ox gall with dilute hydrochloric acid for several hours. The bile acids are thus decomposed:—Taurine and glycocine combine with the hydrochloric acid, and remain in solution, cholic acid separating as a resinous mass. Filter the fluid, evaporate the filtrate to dryness, extract the residue with absolute alcohol to remove the glycocine-hydrochlorate, dissolve the residue in water, and allow it to stand and crystallize. In order to purify it, dissolve it in spirit, precipitate it with lead acetate, decompose the precipitate with hydrogen-sulphide, filter, evaporate the filtrate to dryness, extract the residue with absolute alcohol, dissolve the taurine which remains in a very little water, and allow it to crystallize. Taurine is soluble in fifteen or sixteen parts of cold water, and in a much smaller quantity of hot water. In cold alcohol it is sparingly soluble, more easily in warm alcohol. It is insoluble in absolute alcohol and ether. Taurine is recognized by its crystalline form, and by its containing sulphur. Its crystals are colourless, transparent, six-sided prisms, with four to six sided pointed ends (fig. 312). Taurine is proved to contain sulphur as follows:—If a crystal is heated on platinum foil, it swells, becomes brown, and fuses, giving

off fumes in which sulphurous acid is recognized by its smell. If the crystals are ignited with sodium carbonate, and a little acid is poured over the residue, hydric-sulphide is evolved. If they are dissolved in caustic potash, and the solution concentrated by boiling, ammonia is given off, and potassium sulphate and acetate left in solution.

146. **Cholic Acid.** ($C_{24} H_{40} O_5$)—*Preparation.*—Boil bile (or solution of glycocholic acid) with strong solution of caustic potash, or hot saturated solution of baryta water for twelve or fourteen hours, precipitate by hydrochloric acid, wash the precipitate with water, dissolve it in a little liquor potassæ, add ether, precipitate by hydrochloric acid, and allow the liquid to stand for several days. The ether causes it to become crystalline, and form quadrilateral prisms with pyramidal ends. Pour off the ether, dry the crystals between folds of blotting paper, dissolve them in hot alcohol, and add a little water until a turbidity just commences. Cholic acid crystallizes out on cooling in tetrahedra. Cholic acid exists in two conditions. In one it is soft and waxy, and somewhat soluble in water; in ether tolerably, and in alcohol very readily soluble. In the crystalline condition it is insoluble in water and ether, but tolerably soluble in alcohol. When heated on platinum foil, it becomes brown, melts and burns, giving off incense-like fumes. Heat, or boiling with sulphuric acid, converts it into resinous-looking substances, choloidinic acid and dyslysin.

* 147. **Cholesterin.**—Cholesterin is not generally prepared directly from bile, but from gall stones, most of which consist chiefly of cholesterin, along with a little bile pigment and earthy salts. *Preparation.*—Extract pulverized gall stones with boiling alcohol, and filter while boiling. Crystals of cholesterin separate from the filtrate when cool. In order to purify it, boil the crystals with alcoholic solution of caustic potash. On cooling they will again separate. Wash the product with cold alcohol, and then with water; dissolve it in a mixture of alcohol and ether; allow it to evaporate. Crystallized cholesterin forms rhombic plates, the corners of which are often broken (fig. 314). It is quite insoluble in water and in cold alcohol. In boiling alcohol it dissolves with ease. Cholesterin may be conveniently prepared from the ethereal extract of gall stones obtained in the preparation of bilirubin by evaporation. The crystals must be purified as above directed. *Reactions.*—(1) Put a few crystals of cholesterin under the microscope; add a drop of a mixture of five volumes of sulphuric acid and one of water, and warm the object-glass gently. The edges of the crystals will acquire a carmine colour. If three parts of acid are used to one of water, the edges are violet, and if it is still more dilute they become lilac and dissolve in the acid. (2) Add to some crystals strong sulphuric acid,

with a little iodine or zinc chloride; they acquire a tint which varies from greenish-blue to violet. (3) Put a drop of concentrated nitric acid on a crystal in a porcelain capsule, and evaporate to dryness at a gentle heat: touch the residue with a drop of ammonia. A deep red colour is produced. (4) Rub up cholesterin with strong sulphuric acid, and add chloroform. A solution varying in colour from blood-red to purple is produced, which, after changing successively into violet, blue, and green, finally disappears.

* 148. **Action of Bile.**—The bile appears to aid the absorption of fat. Lenz, Bidder, and Schmidt found that, after ligature of the gall duct, a dog absorbed less fat than before, and that the chyle in the thoracic duct contained very little fat. They calculated the amount absorbed by comparing the quantity of fat eaten with the amount passed with the fœces. The bile emulsionizes fat, as can be seen by shaking a little oil with it. It is doubtful, however, whether it is to this property that the absorption is due. In forcing oil through animal membranes, or filter-paper either by pressure or by suction, it passes with much greater facility if it has been previously mixed with bile.

149. **Bile precipitates Syntonin and Pepsin.**—Digest a piece of fibrin with artificial gastric juice, and then add a large quantity of bile to it; a precipitate is at once produced. Filter, put another piece of fibrin in the filtrate, and acidulate with hydrochloric acid to the proper degree. The pepsin having been precipitated, the fibrin is not digested. Unless the quantity of bile is large, the whole of the pepsin will not be thrown down. It is not known what purpose is served by the precipitation of the chyme by the bile in the duodenum. In the stomach the presence of bile must be injurious.

150. **Secretion of Bile.**—The secretion of bile goes on constantly, but is more rapid at one time than another. It is accelerated after taking food, usually attaining its maximum from two to four hours after each meal. The secretion is observed by tying the gall duct and introducing a cannula into the gall bladder. A detailed account of the method of performing this operation on dogs is given by Rutherford and Gamgee in the report of the British Association for 1868. The principal facts may be demonstrated in the guineapig, as follows:—

** 151. **Mode of Producing Biliary Fistula in Guineapigs.**—Chloroform the animal and secure it on the rabbit-support. Make an incision from an inch to an inch and a quarter long through the abdominal parietes in the linea alba from the xiphoid process downwards. The pyloric end of the stomach is thus exposed. Pull gently on the stomach until the duodenum is brought into view. The part corresponding to the superior transverse part in man forms a loop with its convexity directed towards the diaphragm, into the top of which convexity the *ductus choledochus* enters. Tie the duct in this situation, then seize the gall bladder with a pair of forceps. It is always full, and cannot be missed if the forceps are passed immediately under the edge of the costal cartilages. Make a small incision into the gall bladder, introduce a cannula, and tie it in. The diameter of the cannula should be from two to three centimeters, and the end to be inserted should have a projecting rim. This can be made very readily by heating the end of a piece of glass tubing of the proper size, and pressing it, while hot, against a flat piece of iron. Sew up the wound, leaving the free end of the cannula outside. The bile in guineapigs is secreted in very large quantities, being as much as 7·3 grammes in an hour per kilogramme of body weight. It contains a very small proportion of solids, about 1·3 per cent. When the bile duct is tied the guineapigs die in less than twenty-four hours, but when it is not tied they will live for a week. *The bile is secreted under a very low pressure.* For estimating this pressure, prepare a manometer by attaching a piece of glass tubing, eighteen inches long, to a wooden or pasteboard scale. Fit an india-rubber tube to its lower end, fill the manometer and tube with water, and close the latter with a clip. Tie the *ductus choledochus* of a guineapig, and secure a cannula in its gall bladder. Having ascertained that the water in the manometer stands at about 100 millimeters above the zero point, place the tube in a horizontal position at the same level as the cannula. Connect the india-rubber tubing with the cannula, and remove the clip. As the bile is secreted, the column of water advances, and the rapidity of secretion is thus indicated. When it reaches 150 millimeters on the scale, raise the tube to a vertical position. If the maximum pressure under which secretion occurs in the animal experimented on be used, the water will descend in the tube, but if not, it will continue to rise.

** 152. **Absorption by the Liver.**—The bile which has been secreted by the liver is re-absorbed either when the pressure is diminished in the blood-vessels, or when it is increased in the bile capillaries (Heidenhain); jaundice may thus be produced in two ways. To show absorption from diminished pressure in the blood-vessels, compress the aorta just underneath the diaphragm. The pressure in the manometer sometimes falls, but as the vena cava and other parts are generally compressed likewise, the result is not constant. To show absorption from increased pressure in the ducts, replace the water in the manometer by aqueous solution of indigo-carmine, taking care that the column of fluid stands several inches above the highest level previously attained by it. The solution is gradually absorbed, muscular tremors occur, and the animal dies just as if water had been injected into the veins. At the same time the surface becomes coloured blue by the indigo-carmine. The experiment enables us to understand how a very slight obstruction to the orifice of the bile duct is sufficient to determine re-absorption, and the production of jaundice.

GLYCOGEN.

153. It would form a marked exception to the economical use of material which we find in the body if the liver, the largest gland in it, had as its sole function the secretion of bile; a fluid of much less importance in digestion than the gastric or pancreatic juices. This, however, is not the case, for, in addition to secreting bile, the liver has the power of forming glycogen, a substance which resembles dextrin in its reactions, and, like it, can be converted into sugar by the action of ferments. It is always present in the liver in larger amount during digestion than during fasting. What the materials from which it is formed actually are is uncertain. Its amount is increased by the use of starchy food; but as it continues to be formed in considerable quantity when the food consists of flesh alone, it is evident that it can be produced from albuminous bodies. In support of its origin from albumin, it has been argued that the increased amount which is met with after the administration of starchy food, is due to the sugar derived from the starch being burnt off instead of albumin, in consequence of which more albumin remains to be converted into glycogen. The experiments of Cyon (if they are to be relied upon) make it probable that urea is formed in the liver. As the amounts of sugar and urea excreted by diabetic patients fed on an animal diet, run parallel with one another, it might be supposed that when the diet is exclusively albuminous, glycogen is formed by albumin or peptones splitting up and yielding glycogen and urea. Again, when the diet consists of starch and sugar, glycogen is formed abundantly, and at the same time a deposit of fat takes place in the liver. From this it might be supposed that the sugar absorbed from the intestine is decomposed so as to yield glycogen and fat. Glycogen seems to be of great importance for cell growth, for it is found wherever this is going on actively, as in new formations, or in embryonic tissues. A remarkable experiment of Hoppe-Seyler shows that it is an ingredient of colourless blood corpuscles so long as they are active, but that when they lose their power of motion their glycogen disappears, and is replaced by sugar.* In early fœtal life, the muscular fibres and lungs contain much glycogen, which subsequently diminishes. The liver and other glands, and the nervous system of the

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* For the details of this experiment *see* Med. Chem. Untersuch., 1871, p. 486.

embryo, contain little or no glycogen; but it is found in large quantities in the placenta. After birth it is confined almost entirely to the liver and muscles. In the latter it seems to have some relation to the work done by them, for the quantity present in them is diminished by activity. The glycogen of the liver does not remain in it long, but is soon converted into sugar, so that the large quantity which is present after a meal is quickly diminished by fasting, and disappears altogether during starvation, while that present in the muscles does not increase so much after food, nor is it so quickly lessened by starvation (Weiss).

Although both the liver itself and the blood contain a ferment which transforms glycogen into sugar, its conversion is probably effected in great measure by the blood, for it takes place more rapidly when the circulation through the liver is quickened. It is uncertain what the use of the sugar in the organism is, but possibly it, as well as glycogen, has something to do with muscular action, since the quantity of sugar (or a substance reducing copper) in blood becomes much diminished in its passage through the vessels of contracting muscles (Genersich). While Bernard considers that the formation of sugar goes on in the liver constantly during life, this has been denied by Pavy, Ritter, Meissner, and Schiff, who hold that it only occurs after death, or under pathological conditions, such as disturbance of the respiration or circulation during life. They base their opinions on the observations that the liver contains little or no sugar when examined immediately after death, and that the blood of the hepatic vein does not contain more sugar than that of the portal or jugular veins. It is quite true that sugar is found only in very small amount in fresh livers; but the smallness of the quantity is in all probability due to the constant circulation through the liver during life, washing the sugar out of it as soon as it is formed (Flint). The statement that the blood of the portal contains as much sugar as that of the hepatic vein, rests on experiments vitiated by the omission to place a ligature on the former while removing the liver, so that the hepatic vein having no valves, the blood from it flowed back into the portal system. When this fallacy is avoided, sugar is found in much larger proportion in the hepatic than in the portal vein. To meet the objection that sugar thus found has been formed after death, blood has been taken from the right side of the heart, or vena cava, and the quantity of sugar it contained compared with a similar specimen of blood from the jugular vein. Every precaution was taken to avoid disturbance of the circulation, yet the sugar in the former was found to exceed that in the latter considerably (Lusk).

** 154. **Mode of demonstrating the Glycogenic Function of the Liver.**—*The Liver contains Sugar which can be removed by*

Washing.—Kill a large rabbit in full digestion, by decapitation with a long knife. Open the abdomen, remove the liver, and place it in a large flat dish, such as is used for photographic purposes. Tie a cannula into the portal vein, and another into the hepatic vein. Pass a stream of water through the portal vein. This may be effected by a syringe; but a more convenient method is to connect the cannula in the portal vein by means of india-rubber tubing with a pressure-bottle containing water. (*See* page 101.) Proceed in every respect as in injecting the liver for anatomical purposes, using a pressure of two or three feet of water. The liquid which flows from the hepatic vein as the water enters the portal vein, will be at first blood, then blood diluted with water, and, lastly, pure water. Collect portions of each of these fluids in small beakers as they flow out. The remainder which is not collected is allowed to run into the dish in which the liver lies. Test each of the fluids for grape sugar. It will be found in the portions first collected, the quantity gradually diminishing as the washing is continued. Eventually it disappears. Allow the stream to flow until none can be detected by any of the tests described in the next paragraph.

As soon as this is the case, disconnect the cannula without loss of time, and cut the liver into three pieces. Mince one of them as rapidly as possible, put it immediately into water boiling briskly, and acidulate it very slightly with acetic acid, to coagulate the albumin. Put another into strong alcohol for a minute or two, pour off the alcohol, and squeeze the remainder of it from the liver. Then cut it up small, cover it with absolute alcohol and let it stand. Allow the third piece of liver to lie on the table. After the liver has been boiled for a few minutes filter the water from it. The filtrate is milky. Test it for sugar. If the operation has been rapidly performed, little or none will be found, showing that all the sugar has been removed from the liver.

Sugar is again formed in the Liver after its removal by Washing.—After the third piece of liver has lain on the table for some time, cut it up and boil it like the first; filter, and test for sugar; in most cases it will be found. As there was none in the other piece, this sugar must have been formed after the liver was cut in pieces.

The Liver contains Glycogen, a Substance which can be changed into Grape Sugar by the action of Ferments.—Take a little of the milky filtrate obtained by boiling the liver which has been already found to contain no sugar. Add to it a little saliva, and let it stand in the water-bath at 35° C. for a few minutes, or warm it gently over a spirit-lamp. Then add liquor potassæ and cupric sulphate, and boil; sugar is found. Evaporate the milky remainder of the filtrate to a small bulk, and add alcohol in excess. A white flocculent precipitate of glycogen is formed.

The Liver also contains a Diastatic Ferment.—From the other piece of liver which has been placed in alcohol prepare a glycerin solution, as directed in § 160. Add some of this to a solution of glycogen, let it remain in the water-bath at 40° C., and test small portions of it from time to time. Sugar will at length be found, but very many hours may be necessary.

155. **Mode of Testing for Sugar in Blood.**—As the albumin and colouring matter of the blood would interfere with the reaction, they must be removed before the test is applied. Bernard's method is as follows:—Put the blood, if pure, in a mortar, and rub it up with a quantity of animal charcoal, sufficient to form a dry paste. Add a little water, rub it up again, and throw the mixture on a filter. The water filters through quite clear, holding in solution any sugar which may be present, and Trommer's test may then be applied to it. If the blood is diluted, agitate it well with sufficient animal charcoal to form a thick paste; filter it, and test as before.

Another method, which is preferable if the quantity of sugar is to be estimated, is to mix the blood with three or four times its bulk of strong spirit, and after allowing it to stand for some time, to filter. The residue is then extracted with much alcohol, and after the addition of the extract to the filtrate, the alcohol is evaporated off and the residual liquid tested. Trommer's test answers for saliva, but in the present case it is inadequate, as many other substances capable of reducing cupric oxide might be present. Other tests are therefore required.

Moore's Test.—Put the solution in a test-tube and add sufficient liquor potassæ or liquor sodæ to make it strongly alkaline. Heat it gently to boiling. If sugar is present in considerable quantity, the fluid will become first yellow, then reddish-brown, and, lastly, dark brown or black; but if there is only a little sugar, the colour will only become yellow or reddish-brown.

Böttchers's Test.—Put the solution in a test-tube, and add to it as much bismuth oxide or subnitrate as will lie on the point of a knife, and a considerable excess of a very strong solution of caustic potash or soda, and boil for some time. If sugar is present, the bismuth oxide will be reduced and become at first grey, and lastly black. If only traces of sugar are present, a small quantity of bismuth must be used, or the whole will not be reduced; if a first trial gives only a grey colour, it should be repeated with a smaller quantity of bismuth.

Fermentation Test.—A solution of grape sugar mixed with yeast should at once ferment and give off carbonic acid. A convenient apparatus for testing this is described by Bernard. It consists of a test-tube, about three inches long, fitted with a tight cork, through which a piece of small glass tubing passes to the bottom. The tube is to be completely filled with the fluid to be tested, mixed with a little yeast, and then put in the water-bath at 35° C. If sugar is present, carbonic acid is given off, and as it cannot escape, it drives the fluid out through the small tube. As the yeast may contain sugar itself, a similar tube should be filled with yeast and water for comparison with the first. The gas may be shown to be carbonic acid by shaking it with baryta water. The fluid which escapes should be collected by means of a piece of india-rubber tubing attached to the upper end of the small tube, and tested for alcohol by boiling it with a little potassium bichromate and sulphuric acid. If alcohol is present the fluid becomes green.

** 156. **Preparation of Glycogen.**—In order to obtain a large amount of glycogen from a liver, the animal must be healthy, and must be killed during digestion, as otherwise the liver would contain but little glycogen. Conversion into sugar after death must be prevented by rendering the ferment which acts on it inactive, as quickly as possible; this is done by heating the liver to 100° C.

Kill a large and well-fed rabbit an hour or two after it has had a full meal, by decapitation with a long knife. Open the abdomen instantly, tear out the liver, chop it into pieces as quickly as possible with a few strokes of the knife, and throw it into a capacious capsule containing water, which is kept briskly boiling by a large Bunsen's burner. The burner must be large, because the liver cools the water into which it is thrown, and unless ebullition be kept up briskly it may be some time before the pieces of liver are heated to 100° C. throughout, in which case the transformation of glycogen into sugar will go on in those parts which are insufficiently heated. Let the liver boil briskly for a short time; then pour the liquid out of the capsule into a large beaker, and put the liver into a mortar. Return the liquid to the capsule, rub the liver to a fine pulp, put it back into the capsule and boil it again. Then filter the liquid and cool the filtrate rapidly, by placing the vessel containing it in iced water. The filtrate contains a considerable quantity of albuminous substances, which must be removed in order to get the glycogen pure. This is best done by precipitating them with potassio-mercuric iodide, as recommended by Brücke. This solution is made by precipitating a solution of mercuric chloride with potassium iodide, washing the precipitate and adding it to a boiling solution of potassium iodide till the latter is saturated.

When the filtrate from the liver is cool, add hydrochloric acid and potassio-mercuric iodide solution to it alternately, as long as they cause any precipitate. Stir the mixture, let it stand about five minutes, and then filter. Add alcohol to the filtrate till glycogen begins to be copiously precipitated, taking care not to add an excess of alcohol, lest other substances be also precipitated. The glycogen is best precipitated when the mixture contains 60 per cent. of absolute alcohol. Collect the glycogen in a filter, wash it, first with dilute alcohol, then with strong alcohol of 90 per cent. (sp. gr. 822), which makes it more easy to separate from the filter. Extract it with ether and dry it rapidly on a flat plate. Instead of separating the albumin from the glycogen by potassio-mercuric iodide, the boiling solution of glycogen may be slightly acidulated with acetic acid and filtered. The filtrate is then quickly evaporated to half its bulk and mixed with its own volume of strong alcohol of 90 per cent. The glycogen is precipitated along with a little glutin. To separate it from this it is boiled with liquor potassæ for an hour or more, neutralized with acetic acid, precipitated with alcohol, collected on a filter, washed first with strong alcohol and then with absolute alcohol till all traces of water have been removed, and then the alcohol displaced by absolute ether. The glycogen remains as a white powder. It is to be quickly dried by spreading it in a thin layer on a warm porcelain plate and passing a current of air over it. If the glutin has not been perfectly removed, or if the water has been incompletely displaced by the alcohol and ether, the glycogen in drying becomes converted into a gummy mass, instead of forming, as it ought to do, a white powder.

* 157. **Properties of Glycogen.**—Glycogen is amorphous, colourless, and tasteless. In water it is readily soluble. The solutions are strongly opalescent, and when concentrated are quite milky. They are, apparently, true solutions, as they pass unchanged through filters and through animal charcoal, and no particles can be observed in them by the microscope. Brücke, however, considers that they are not true solutions, but merely suspensions of particles of glycogen swollen up in the fluid. The opalescence disappears on the addition of caustic alkalies, although the alkali does not destroy the glycogen. In alcohol and in ether it is insoluble. It contains no nitrogen. When burnt on platinum foil, it does not give off the peculiar smell of nitrogenous bodies, nor does it leave any ash.

Glycogen is coloured red by solution of iodine. The best solution for this purpose is made by putting a little iodine in water and adding potassium iodide very gradually to it, with constant agitation, until the fluid assumes a wine-red colour. If caustic potash is added to a solution of glycogen, and then a drop of cupric sulphate, the copper oxide is redissolved. The oxide is not reduced on boiling.

158. **Influence of Food on the Amount of Glycogen in the Liver.**—If two rabbits, one of which is fed abundantly with corn,

the other sparingly with green food, are kept otherwise in the same conditions and killed at the same period of digestion, it is found that the liver of the former contains much more glycogen than that of the latter.

** 159. **Conditions which determine the Conversion of Glycogen into Grape Sugar.**—Glycogen can be changed into dextrin and grape sugar :—

1. *By Ferments.*—Take a watery solution of glycogen and mix some saliva with it. Put the mixture into two test-tubes and place them in the water-bath at 37° to 40° C. Take out one immediately after the milkiness of the solution has disappeared. Add alcohol to it; a precipitate of dextrin is formed. Filter, and wash the precipitate with alcohol. Put the precipitate in water; it becomes transparent and dissolves, forming a solution perfectly free from opalescence. Test a little of the solution with liquor potassæ and cupric sulphate; no reduction takes place on boiling. To another portion add iodine solution; a red colour like that of glycogen appears. Test the alcoholic filtrate with liquor potassæ and cupric sulphate: it is reduced. This shows that the glycogen has been converted partly into dextrin and partly into grape-sugar by the salivary ferment. Let the other test-tube stand for some time in the water-bath. Add alcohol. If it has stood long enough, no precipitate is produced. Test it. On applying Trommer's test a great reduction of cupric oxide will occur. This shows that the glycogen has been entirely converted into sugar by the prolonged action of the salivary ferment.

Blood contains a Ferment which converts Glycogen.—A ferment possessing the same action is contained in the blood. Add a little blood to a solution of glycogen, and let it stand for some time at 37° C. Then remove the albumin and test for sugar in the manner already described.

2. *By Acids.*—Mix a solution of glycogen with dilute hydrochloric or sulphuric acid and boil. Then add liquor potassæ in excess and copper sulphate, and boil; sugar is found. All specimens of glycogen can be converted into sugar by acids, but they are not all alike in their behaviour to ferments, some specimens requiring a longer time than others.

160. **Separation of a Diastatic Ferment from the Liver.**—Cut off the head of a rabbit and remove the liver as quickly as possible. Mince it and wash it with water several times to remove the blood. Then squeeze it tolerably dry, put it into absolute alcohol, and let it remain for twenty-four hours. Filter off the alcohol, wash the liver with alcohol, and then put the mass for several days in glycerin. Filter it through muslin. The filtrate is free from sugar, but contains a ferment which converts glycogen and starch into sugar. Take a little of the glycerin ex-

tract in each of three test-tubes; put into one a little glycogen, and into another a little starch paste, and let them stand for a quarter or half an hour. Then test all three for sugar with copper sulphate and potash. No sugar will be found in the tube containing the glycerin extract alone, the sugar found in the liver immediately after death having been removed by the alcohol before the glycerin was added. Both the other tubes will contain sugar. Diluting the glycerin extract does not alter the effect.

After the Ferment has been extracted by Glycerin, the Mass still contains Glycogen.—Extract the mass several times with fresh glycerin. Take two test-tubes: then introduce a little of it, with water in each, and let them into two test-tubes. Test one of them for sugar: none is found. Add to the other one a little of the glycerin extract, which has already been found to contain no sugar, and let it stand at 40° C. for some time, after which it will be found to contain sugar. A similar ferment can be extracted from bile by precipitating it with alcohol, washing the precipitate with alcohol on a filter, and then extracting it with glycerin in the way already mentioned (Von Wittich).

161. Glycosuria.—It is still disputed whether sugar is a normal constituent of the urine or not. But in the diseased condition to which the term *Diabetes Mellitus* is applied, it appears in considerable quantities. Bernard first showed that its appearance in the urine can be induced by certain lesions of the nervous system, and finding that they caused at the same time dilatation of the vessels of the liver, he attributed the appearance of the sugar to the increased circulation through that organ. His views have lately been confirmed; the nervous mechanism by which the vessels become dilated has been discovered by Cyon and Aladoff, from whose researches it appears that the vasomotor nerves of the hepatic vessels pass from the vasomotor centre in the medulla oblongata down the cervical part of the spinal cord, which they leave at its lower end. Thence they accompany the vertebral arteries to the last cervical ganglion, finding their way by the two fibres, which pass in front and behind the subclavian artery (forming the annulus of Vieussens) to the first dorsal ganglion. Thence they proceed in the gangliated cord of the sympathetic and the splanchnic nerves to the liver. When these vasomotor fibres are severed, either by dividing the fibres on the vertebral artery or those forming the annulus of Vieussens, or by extirpating the third cervical or first dorsal ganglion, the hepatic vessels dilate, and diabetes occurs. It is of great importance to notice that section of the sympathetic cord or the splanchnic nerves does not produce diabetes, although the vasomotor nerves of the liver are thus divided. The reason of this probably is that the vasomotor nerves of the intestine being divided at the same time, so much blood goes to the intestinal vessels that the circulation in the liver is not increased. The vessels can be dilated reflexly by irritating the central ends of the cut vagi, or the roots of the vagus in the fourth ventricle. Section of the splanchnics or sympathetic cord prevents the occurrence of diabetes when the fourth ventricle is afterwards punctured, but does not remove it when already present. Diabetes may also be produced by the inhalation of carbonic oxide (Schmiedeberg), chloroform, or nitrite of amyl, or by the injection of curare. As regards carbonic oxide, it has been ascertained that the action is not prevented in the dog by section of both splanchnics, but in rabbits it does not produce diabetes at all (Eckhard).

Increased proportion of sugar in the blood determines glycosuria. To show this, expose the jugular vein in a healthy rabbit, having first weighed it and ascertained that its urine is free from sugar. Then slowly inject 5 to 10 per cent. solution of grape sugar into the vein. About two grammes of sugar should be used for every kilogramme of body weight. Sugar is found in the urine shortly after, but next day it will have disappeared. It has been found that if the amount of sugar in the blood does not exceed a half a gramme for each kilogramme of body weight, it may not appear in the urine.

** 162. **Production of Glycosuria by Puncture of the Floor of the Fourth Ventricle.**—The part of the fourth ventricle the puncture of which is followed by the most abundant appearance of sugar in the urine is limited superiorly by a line joining the origin of the auditory nerves, and inferiorly by one joining the origins of the vagi; a puncture higher up, or to either side, may, however, produce more or less glycosuria. It has been ascertained by Bernard that it is essential to the result, that the olivary fasciculi should be injured, and that it is not produced by injury of the superficial, *i.e.*, posterior sensory layers. The instrument used for puncturing the ventricles consists of a small steel chisel (*see* fig. 315), about four millimeters broad, and having a style in the middle which projects about two millimeters beyond the cutting edge. This chisel is pushed on through the occipital bone and the cerebellum until its further progress is arrested by the point coming in contact with the basilar process of the occipital bone. In this way the edge of the chisel is prevented from injuring the anterior motor fibres of the medulla, and thus producing a disturbance of the motor functions which would complicate the experiment.

Mode of Operation.—Place a rabbit in the prone position on Czermak's rabbit-support, and fix the head to the upright at the side. Feel for the occipital protuberance, and make an incision over it about half an inch long. Fix the point of the chisel in the middle line of the skull just behind the protuberance, and bore through the bone, moving the handle of the instrument from side to side, in order to assist its passage, but not pressing with too great a force. When the skull has been penetrated, push the chisel downwards and forwards through the cerebellum in such a direction as to cross a line joining the two auditory meatûs (*see* fig. 316) until it is stopped by the basilar process, and then gently withdraw it. Remove some of the urine in half an hour or an hour afterwards (§ 138), and test it for sugar.

SECTION IV.—DIGESTION IN THE INTESTINES.

PANCREATIC JUICE.

163. Pancreatic juice may be obtained either by a temporary or permanent fistula. It is usually stated that the secretions from these two kinds of fistula differ much from each other, a normal juice being obtained only from a temporary fistula, while that yielded by a permanent one is watery and destitute of some of the properties possessed by the other. Ludwig and Bernstein, however, have, by an improved method of making a permanent fistula, succeeded in obtaining a normal juice from it also.

164. **Method of making a Temporary Fistula.**—In the dog there are two pancreatic ducts, one of which opens into the duodenum along with the *ductus choledochus*. The other duct, which is larger, and enters the duodenum about two centimeters below the one first mentioned, is exclusively employed for the operation. It is not necessary to ligature the first. Bernard prefers for the purpose large dogs, sheep dogs being best, as they are less subject to peritonitis than others. Five or six hours before the operation, the animal should get a large meal of bread and meat. The operation, which must be performed as quickly as possible, consists in laying the dog on its left side, and making an incision five centimeters long in the right hypochondrium from the projecting point of the last false rib downwards, parallel with the linea alba. The bleeding should be stopped before the peritoneum is opened. The duodenum lies opposite the wound. As soon as it is exposed it is drawn out, and the pancreatic duct looked for about two centimeters below the *ductus choledochus*. The part of the pancreas in which the duct lies is generally closely attached to the duodenum, and somewhat overlaps it. The largest and lowest of the bundles of vessels which pass from the duodenum to the pancreas, lies over the duct. These vessels are to be pushed aside, and a thread passed under the duct, which is recognized by being larger and paler than the vessels. Care must be taken not to injure the vessels and cause bleeding, and the pancreas must be pulled or pressed as little as possible. The duct is opened with scissors, and a plain silver cannula, about

five millimeters in diameter, and 10 or 12 centimeters long, pushed into it up to its first division, which is generally visible; the ligature is then tightened; another thread is passed through the serous coat of the duodenum, and the cannula fixed to the intestine by it. The ends of these threads, and the end of the cannula, are kept outside the wound, the duodenum returned to the abdominal cavity, and the wound closed by first sewing together the muscles, and then the skin. A small india-rubber bag, furnished with a stopcock, is then tied to the outer end of the cannula, emptied of air, and the stopcock closed. The juice then collects in it, and is drawn off by the stopcock (*see* fig. 317). Generally, it flows abundantly; but if it does not, a little ether should be injected into the stomach by a stomach-pump. The juice may be collected for several hours; but after the expiration of twenty-four hours, the character of the secretion changes. In a few hours more, the cannula and threads should be gently drawn out. The wound generally heals quickly.

165. **Method of making a Permanent Fistula.**—For permanent fistulæ, Ludwig and Bernstein choose small dogs, as in them the duodenum is more easily reached from the middle line, and is not drawn so far from its natural position by the fistula as in larger animals. The dog must be kept fasting on the day of the operation, as the pancreatic vessels are full during digestion, and bleed easily. Narcotize the animal by injecting opium into the tibial vein, and open the abdomen by an incision about two centimeters long in the linea alba, midway between the ensiform cartilage and the umbilicus. The duodenum is then searched for, and drawn out of the wound along with the attached pancreas, and a thread looped round the duct. Instead of then putting in a cannula, a piece of lead wire is inserted into the duct, so that one end of it passes into the intestine and the other into the gland to a considerable distance. The middle part of it is twisted together, and projects through the wound. Owing to the T shape thus given to the wire, it cannot either slip out or move about in the duct; but wire being chosen which does not fill it up, the flow of the juice is not hindered. Three threads having then been passed through the wall of the duodenum near the duct, the intestine and omentum are replaced in the abdomen, and the duodenum fastened by the threads to the abdominal wall. The wound is then sewed up, care being taken that the twisted part of the lead wire passes through the wound. Twenty-four hours after the operation, the stitches are taken out, but the wire left in. In two or three days afterwards the juice can be collected. For this purpose, the animal must be supported by two straps, which

pass under its belly, and are attached to a horizontal bar hung from the roof by a cord and pulley. The dog is thus suspended over a table at such a height that it can barely touch it with its toes, in which position it remains perfectly still. A funnel is then attached under the fistula, and the juice collected in a glass below.

The normal juice obtained from a temporary fistula is a colourless transparent tenacious fluid, with a strongly alkaline reaction. When cooled under 0° C., a coagulum separates from it. The juice from permanent fistulæ is more watery, and yields no coagulum when cooled. In the former, it often contains about 10 per cent. of solids, but the amount may be as low as 2 per cent.; and in the latter, the percentage is frequently from 2 to 5. Their amount is determined in the manner described in § 74. Pancreatic juice contains an albuminous body, an alkali-albuminate, leucine, tyrosine, fats, soaps, inorganic salts, and three ferments. One of these converts starch into sugar, another splits up fats, liberating fatty acids, and the third converts albuminous bodies, first into peptones, and then into leucine and tyrosine. On account of the presence of this third ferment, the reactions of the juice, after it has been allowed to stand, differ from those which it presents when fresh, the albumin of the fresh juice itself being digested by the ferment in it, and yielding peptones, leucine, and tyrosine. When fresh juice is heated to 72° C., the albumin coagulates, and after the coagulum has been separated, acetic acid precipitates the alkali-albuminate. The watery extract of the gland may be used for showing many of the properties and actions of pancreatic juice, instead of the juice itself.

** 166. **Artificial Pancreatic Juice.**—For this purpose, the pancreas from an animal killed in full digestion must be employed. Take the pancreas of an animal which has been killed about six hours after a full meal. Wash off the blood, cut it into moderately small pieces, pour about four times its weight of water at 25° C. over it, and let it stand for two hours in the water at that temperature, above which it must not be allowed to rise more than four or five degrees at most. Filter it first through linen, and then through paper. The filtrate generally has an acid reaction from the presence of fatty acids, liberated by the ferment from the fats in the pancreas, and is opalescent from the presence of fat in a state of emulsion. Boil a little of the fluid; a precipitate of albumin is formed. Filter, and neutralize by acetic acids, and a further precipitate of alkali-albuminate will be produced. The presence of leucine and tyrosine may be shown by removing the albumin by boiling and acidulating, and then separating them as described in § 35. To show that leucine is present in the juice as secreted, and is not due to changes in it afterwards, it must be received in alcohol as it flows from the fistula.

* 167. **Glycerin Solution of Pancreatic Ferments.**—After cutting up the pancreas, as in the previous experiment, lay it for a day or two in absolute alcohol, and after expressing the alcohol let it lie several days in glycerin, then filter.

** 168. **Actions of Pancreatic Juice.**—*It emulsionizes Fat.*—Shake up some of the watery extract with olive oil, an emulsion is formed. This is due to the albumin it contains, for by adding liquor potassæ to the mixture so as to dissolve the albumin, and shaking, the drops of fat may be made to run together again.

2. *It decomposes Fats, liberating Fatty Acids.*—The extract of pancreas contains fat: hence when it is kept for an hour in the water-bath at 40° C., without any addition, its acid reaction increases. To show its action on fats, carefully neutralize some of the watery extract and add to it a little olive oil or fresh butter, whose reaction must also be neutral. Put the mixture in the water-bath for some time, put a drop from the bottom of the tube on blue litmus paper and let it run off. A red and somewhat greasy spot is left.

3. *It converts Starch into Sugar.*—Mix some of the extract with starch mucilage and let it stay for some minutes in the water-bath at 40° C.; then apply Trommer's test, and sugar will be found.

4. *It digests Fibrin, forming Peptones, and afterwards decomposes them, Leucine and Tyrosine being produced.*—Before dissolving boiled fibrin, the pancreatic juice converts it into a soluble albuminous substance, very much like raw fibrin. This is then dissolved and is present in solution, either as albumin, coagulable by heat, or as an albuminate. The dissolved albumin is next converted into peptones. If the digestion is allowed to go on, the quantity of peptones in the solution diminishes, while that of leucine and tyrosine increases. Bodies which give the reaction of naphthilamine and indol (Kühne) are also formed, and when the digestion goes on for a long time the indol is formed in considerable quantities, and emits a most disagreeable fœcal odour, which was attributed to putrefaction till Kühne showed its true nature. Boil several bits of fibrin in a large test-tube, pour off the water, add artificial pancreatic juice or glycerin extract of pancreas, and put the tube in the water-bath at 40° C. At first it will not be altered, but after two hours or more the bits will be found to be easily torn by stirring, and the smaller ones will disappear, and if two or three are taken out and washed with water they will be seen to be corroded, not swollen as in gastric juice. To show that the coagulated fibrin has been converted by the pancreatic juice into a body resembling raw

fibrin in its properties, put a bit into 0·1 per cent. of hydrochloric acid. It dissolves very quickly, forming a solution of syntonin. Rub up a second bit with 10 per cent. salt solution, and filter. The filtrate contains albumin in solution. Add nitric acid to one portion of it and boil another; a precipitation occurs in both. If boiled fibrin is tested in the same way, it is found to be insoluble in these reagents. Even raw fibrin is much less soluble than the boiled fibrin which has been acted on by pancreatic juice.

Take part of the solution of fibrin in pancreatic juice and boil it. Neutralize another portion with acetic acid; a precipitate is formed in both. Let the rest stand for two or three hours longer, then acidulate it with acetic acid and boil, to coagulate any albumin present. Filter. Evaporate the filtrate at 60° to 70° C., and add alcohol to it while still hot, till the peptones are precipitated. Let it stand for twenty-four hours and filter. Dissolve the precipitate of peptones in water and apply the tests given in § 118. Evaporate the filtrate to a moderately small bulk and let it cool. Tyrosine crystallizes out. Pour off the mother liquor, evaporate it to a small bulk, and leucine will crystallize out. In order to purify the tyrosine, put it on a filter and wash it, first with ice-cold water till the filtrate is colourless, and then with spirit, next with absolute alcohol, and lastly with ether. To purify the leucine, put the crystals on a filter, which must be allowed to stand in a cool place until not a drop more runs from it. Then wash it, first with ice-cold water until the filtrate is colourless, next with common alcohol, then with absolute alcohol, and lastly with ether. It is of great importance that the mother liquor should be allowed to drain away completely before the washing, as otherwise the crystals would dissolve in the water used. Test the mother liquor for naphthilamine and indol. In testing for the former, dilute naphthilamine is indicated by the appearance of a rose-red colour when chlorine water is added gradually to the mother liquor diluted with water. To prove the presence of indol, dilute some of the mother liquor, boil it in a test-tube, add a little dilute sulphuric acid and a drop or two of a dilute solution of a nitrite; or of *very* dilute nitrous acid, a red colour is produced. The dilute nitrous acid for this purpose may be conveniently obtained by boiling a small piece of grape sugar with nitric acid in a test-tube, and when the tube is filled with red fumes emptying out the acid and filling the test-tube with water.

* 169. **Separation of the Pancreatic Ferments from the Glycerin Extract.**—Precipitate the glycerin extract by absolute alcohol; filter; treat the precipitate again for a week or two with glycerin, and filter; let the filtrate fall drop by drop into

a tall cylinder filled with absolute alcohol. The ferment is precipitated in white flocculi. After the precipitation is complete, let it stand one or two days under a mixture of alcohol and ether. Filter by means of Bunsen's pump, and wash several times with alcohol and ether. Let the precipitate dry over sulphuric acid, and then pulverize it (Hüfner).

170. **Isolation of the Pancreatic Ferments.**—Two of the pancreatic ferments have been separated by Danilewsky; but that which splits up fat is removed or destroyed by the magnesia he employs. His method is as follows:—Wash the pancreas of a dog which has been killed six hours after a full meal thoroughly from blood, and rub it to a fine pulp in a mortar, with about a quarter of its bulk of magnesia, and four times its bulk of water. Put the mixture in a beaker, and let it stand for two hours at 25° in the water-bath. After it has cooled, and the pulp and magnesia have nearly subsided, filter the fluid, but do not put the sediment on the filter, as it chokes it, and, at the same time, partly passes through. Neutralize the filtrate with dilute hydrochloric acid, and put it into a flask large enough to hold three times as much. Pour into it without stirring $\frac{1}{4}$–$\frac{1}{3}$ of its volume of thick collodion, and shake it sharply for several minutes, and repeat the shaking several times. Pour the liquid into a large beaker, and stir it constantly, so as to favour the escape of ether and prevent the collodion from separating in large lumps. When the collodion presents the appearance of small rounded granules, filter through linen, and evaporate the ether from the filtrate *in vacuo*. Then treat the liquid with collodion a second time, filter through the same piece of linen, unite both filtrates, and put them aside. . . . . (*a*)

Wash the precipitate several times with spirit (60 to 70 per cent.), and dry it without removing it from the linen between double folds of blotting paper. Spread it out with a spatula, and leave it exposed to the air till it is dry. Then shake it in a tall narrow glass with ether, to which a little absolute alcohol has been added, till the precipitate is dissolved and a turbid solution obtained. Let it stand for two days, and then decant the turbid fluid from the precipitate, and after diluting it with ether, pour it into two tall glasses and let it stand for several days till a new precipitate subsides. Collect that which then remains suspended by filtration through Swedish paper. Remove the collodion from each precipitate by agitating it with ether several times, and then dry it *in vacuo*. Treat the yellowish residue (which consists of an admixture of coagulated albumin with that pancreatic ferment which acts on fibrin) with cold water, and filter. The ferment will be dissolved and the albumin left. Test the digestive power of the filtrate on a bit of fibrin.

Evaporate the filtrate (*a*) *in vacuo,* filter from the collodion that separates, heat to 43°–44° C. in a water-bath, in order to separate an albuminous body contained in it which coagulates at this temperature. Filter; evaporate the filtrate *in vacuo* to one-sixth of its bulk, and add a large quantity of absolute alcohol. It is advisable to let the precipitate thus produced remain under the alcohol for some days, as it is thus rendered more insoluble in water. Collect the precipitate on a filter, and wash it several times with strong spirit. Then treat it with a mixture of one part of strong spirit and two parts water, in order to dissolve the ferment and leave the albumin. Filter; evaporate the filtrate to dryness *in vacuo,* and dissolve the residue in water. The solution converts starch quickly into sugar, and digests fibrin, but not very quickly, the ferment having this latter action not having been completely removed by the collodion. It contains also leucine and tyrosine, but the greater part of these may be removed by dialysis at 4° C. The ferment should then be dried in order to keep it.

171. Preparation of Tyrosine by Pancreatic Digestion.— Take out the pancreas of an animal which has been fed five or six hours before being killed, weigh it, cut it in small pieces, and rub it up with ten times its weight of raw fibrin, and add to the whole twelve or fifteen parts of water at 45° C. Keep the whole at this temperature for four to six hours, stirring frequently; then add a little acetic acid, and boil to coagulate albumin. Filter through a piece of linen, and evaporate the filtrate quickly to a syrup. Pour it, while still hot, into a flask, and add strong spirit to it till a distinct flocculent precipitate occurs. Let it cool; filter, and distil the filtrate till it forms a thick pulp while still warm. Let it stand for a day in the cold to allow complete crystallization to take place; then throw it on a filter, and let the mother liquor drain completely away; wash the residue with a little cold water, and then put it into a large quantity of water at 50° C., which will dissolve the leucine and leave the tyrosine. Dissolve the tyrosine in hot water, let it crystallize out, and then dissolve it again in ammonia and recrystallize.

INTESTINAL JUICE.

172. Intestinal juice was first obtained pure by Thiry, who divided the jejunum or ileum in two places at a distance of 10 to 15 centimeters from each other, sewed up one end of the piece

thus isolated, and attached the other to the wound in the abdominal walls. The short *cul-de-sac* of intestine formed in this manner remained attached to the mesentery, and its vessels and nerves being uninjured, it yielded a normal secretion which could thus be collected without admixture with other digestive secretions and products. The continuity of the alimentary canal was restored by sewing together the divided ends of intestine.

173. **Intestinal Fistula.**—The method employed by Thiry has been somewhat modified by Paschutin, who prefers the duodenum and the beginning of the jejunum, a part of the small intestine which yields a very active secretion. In making a fistula by his method, the hair must be carefully removed from the skin, and an incision 3 to 5 centimeters long made in the *linea alba*. The duodenum is drawn out and two stout ligatures passed round it about two and a half centimeters beyond the spot where it separates from the pancreas. The ligatures having then been separated from each other and tightened, the intestine is divided between them. The upper end of the duodenum is then replaced in the abdomen.

The next step in the operation is to divide the jejunum in a similar manner. The most obvious method of accomplishing this would be to follow the intestine down to the point at which the second division is to be made. This is, however, rendered impossible by the extreme shortness of the mesentery at the point where the duodenum ends in the jejunum. It is, therefore, necessary to find the jejunum independently, by following the intestine upwards from any loop which may present itself in the wound. It is obvious, however, that before this can be done, the operator must find out in what direction the intestine must be followed. For this purpose, the loop being held tight between the finger and thumb, a quantity of half-per-cent. salt solution is injected into the lower cut end of the duodenum, by a syringe with a conical nozzle, which is passed through the tightened ligature. As the fluid passes downwards until it meets the obstruction presented by the fingers, the upper part of the loop is at once recognized by its becoming full. The distended gut is then followed up till the beginning of the jejunum is reached, which is recognized by the mesentery becoming shorter. Two ligatures are passed round it, and the intestine divided between them as before. The under end is replaced in the abdomen, and the upper end closed by sutures so as to form the *cul-de-sac*. Before doing so, the bundle of mesenteric vessels leading to the part constricted by the ligature must be compressed between the finger and thumb, while the constricted part is cut off. As it is necessary that the serous surfaces should be in apposition, the mucous membrane, which is turned outwards by the contraction of the muscular coat, must be first turned inwards, and the closure effected by sutures, applied as shown in fig. 318. The closed end is then replaced in the abdomen, and the continuity of the intestine again restored by joining the cut ends of the duodenum and jejunum. In doing this, the two ligatures, with the parts constricted by them, must be cut off in the manner previously directed. The ligatures applied to the vessels should include a little of the adjoining intestinal wall, so as to give them a firmer hold. The two cut ends are now brought into apposition, and the ligatures firmly tied together so as to retain the ends in their proper relation, and held in the hand of an assistant. The first stitch is put through the intestines in such a way as to include both bundles of vessels, and should be drawn very tight and tied, so that it not only unites the ends, but serves as an additional ligature for the vessels.

To prevent the ligature from cutting the intestine, it should either be made of very thick soft silk, or of two or three fine ligatures used together. Five or six similar stitches made at a little distance from each other on each side of the first are sufficient to join the mesenteric edge of the two pieces of intestine, which then lie with their axes parallel (fig. 318). To complete the junction, the two ends must be brought into the same straight line and sewn together. The application of the final sutures is a matter of considerable difficulty, principally on account of the tendency of the mucous membrane to become everted. The mode of applying the sutures so as to accomplish this object, will be at once understood by a reference to fig. 319. Several threads, each with a needle at each end, must be prepared. For the first suture, one needle enters the intestine from its serous aspect at *a*, and is brought out at *b*, the other enters at *a′*, and is brought out at *b′*. The two ends, *b* and *b′*, are drawn tight and knotted together. For the second suture, one needle enters at *b*, and is brought out at *c*, the other enters at *b′*, and is brought out at *c′*, and so on. To conclude the operation, the wound in the abdominal wall is brought together by sutures, and the open end of the *cul-de-sac* sewn into it. It is also desirable that the junction of the divided intestine should be secured to the wound by a suture, in order to prevent the induction of general peritonitis by its locomotion.

** 174. **Artificial Intestinal Juice.**—Remove the small intestine from a pig, dog, or rabbit, as soon after death as possible; put a ligature round its upper end, attach the lower end to the tap, and fill it with water under pressure. Close the lower end by compressing it between the finger and thumb, and raise, first the one end, and then the other, so that the water may loosen the contents of the intestine from its walls. Empty out the water, and repeat the process three or four times, until what flows from the intestine is either transparent or only slightly opalescent, and is not at all tinged with bile. Five minutes' washing is generally sufficient to cleanse the intestine thoroughly. It should not be continued longer than is necessary, as otherwise a great part of the intestinal ferment may be removed. Slit up the intestine, and separate the mucous membrane from the muscular layer. Cut the mucous membrane into small pieces with scissors, or rub it up in a mortar with sand or pounded glass, then mix it with three to six times its bulk of water, and let it stand for a quarter of an hour to two hours. Filter the infusion through muslin, and then through paper.

** 175. **Actions of Intestinal Juice.**—1. *It converts Starch into Sugar.*—Add a little of the artificial juice to some starch mucilage, warm it, and test for sugar as described in § 77 or 155. The mucilage and juice alone should also be tested, in order to be sure that neither of them contains sugar. 2. *It converts Cane Sugar into Grape Sugar.*—Dissolve some cane sugar in water, and apply Trommer's test to a portion of the solution. No reduction of the copper will occur as it would do if grape sugar were employed. Add some artificial intestinal juice to another portion of the solution. Let it stand at 40° for a short time, and then apply Trommer's test; a reduction of the copper will take place. A similar conversion of cane into grape sugar is produced by boiling with acids, as may be shown by boiling a little of the syrup with dilute sulphuric acid, and then applying Trommer's test.

* 176. **Moreau's Experiment.**—*When all the Nerves going to a part of the Intestine are divided, it secretes a very large Quantity of a watery Intestinal Juice.*—This is shown by letting a large dog fast for at least twenty-four hours, so that its intestines may be empty. It is then put under chloroform, an incision made in the *linea alba*, and a loop of intestine drawn out. Two ligatures are tied firmly round it at a distance of four or five inches apart, so that the piece of bowel between them is completely isolated from the rest of the intestine. All the nerves in the mesentery belonging to this piece are then carefully divided, leaving the vessels uninjured. Another ligature is then tied round the intestine on each side of the first two, and about four or five inches from them, so that a piece of intestine similar to the first is isolated on each side of it, but the nerves going to them are left untouched. The intestine is then returned to the abdominal cavity, the wound sewn up, and the animal left for four or five hours. It is then killed, and the intestine exa-

mined. The part of which the nerves have been divided is found perfectly full of fluid, while the piece on each side of it is empty. The fluid contained in the distended loop has been ascertained by Kühne to resemble in composition diluted intestinal juice.

177. **Movements of the Intestine.**—The influence of the nervous system on the movements of the intestine has not yet been completely investigated. Peristaltic action is in all probability produced by the ganglia in the intestinal walls, as it continues in an excised portion; but it may be increased by the action of the vagi, and lessened or arrested by the splanchnics. The ganglia are stimulated and movements excited by the presence of venous blood in the intestinal vessels (Maier and Von Basch), or their distension by arterial blood (Nasse). The splanchnics are inhibitory nerves for the intestine, and its movements are arrested by their irritation (Pflüger and Westphal). At a certain period after death, however, they excite movements (Ludwig and Spiess). It is uncertain whether they exert an inhibitory action directly on the ganglia as the vagus does in the heart, or act only indirectly through the absence of blood which they produce by causing contraction of the vessels. For a description of the method of showing the action of the splanchnics, *see* Ludwig and Spiess; Sitzungsberichte der Wiener Academie, xxv. 1857, p. 580. Their inhibitory power is said by Kölliker to be destroyed by curare, and the writer has been unable to observe it in several experiments on animals narcotized by chloral. Irritation of the vagi causes movements of the intestine, beginning in the stomach. This occurs only occasionally when one or both splanchnics are intact, but almost invariably after both have been divided (Houckgeest). In performing this experiment, as well as others on the intestine, it is advisable to employ Sanders-Ezn's method, of opening the abdomen under ¾ per cent. salt solution warmed to 35° C., in order to avoid the irritation to the intestines which is occasioned by their exposure to air. For this purpose, a bath of tin or zinc, 32 inches long, by 9½ broad, and 8½ deep, provided with a Geissler's regulator at one end, is used. Into this thirty-five litres of water at 35° C. are poured, and sufficient salt added to make a ¾ per cent. solution. Instead of measuring out the water each time, it is more convenient to mark on the sides of the bath the height to which it should be filled. The animal being laid on a piece of board with Czermak's holder attached to it, instead of the usual support, is placed in the bath, and the lower end of the board is kept immersed by attaching a weight to it. For detailed experiments with this method, *see* Houckgeest Pflüger's Archiv., vi. p. 266.

CHAPTER XXXVIII.

THE SECRETIONS.

SECTION I.—MILK.

178. **Characters of Milk.**—Newly-drawn milk is an opaque fluid of a white or yellowish-white colour. Its colour and opacity are due to its being an emulsion, *i.e.*, to its consisting of little globules of fat suspended in a solution of albumin, milk, sugar, and organic salts. Each globule of fat is covered by a thin coating of casein. When the milk is allowed to stand, the fat globules, being lighter than the fluid in which they swim, rise in great part to the top, and form cream, and the lower part of the fluid often acquires a bluish tinge. A similar separation also takes place in the milk gland itself, so that the milk last drawn is richest in cream. The globules of fat are prevented from uniting by the thin albuminous coating which surrounds each; but when this is broken by agitation, they coalesce, forming butter. Changes also occur in the milk, sugar, casein, and fats of the milk, more or less quickly, according to the higher or lower temperature to which it is exposed. The milk-sugar becomes converted, apparently through the agency of a ferment, into lactic acid. This gives the milk an acid reaction, and precipitates the casein, causing the milk to curdle. The coagulum, or curd, encloses the fat globules. The liquid from which it is separated, a solution of milk, sugar, and salts, is known as whey. The curd, when completely separated from the whey, is called cheese.

Microscopical Examination. — Examine milk under the microscope. It will be seen to consist of a colourless fluid, containing large numbers of minute fat globules. Add a drop of acetic acid, so as to dissolve the coating of casein: the globules will coalesce. Besides these globules, cells containing much fat may be seen, and also masses of fat similar to those within the cells, but destitute of an envelope. These cells are found much more frequently in the milk (called colostrum) which is secreted for the first few days after parturition, and they have, therefore, received the name of colostrum corpuscles. They sometimes exhibit contractile movements.

Reaction.—The reaction of human milk is always alkaline, and that of cows' milk is generally so. Free lactic acid always exists in the fresh milk of the carnivora, and occasionally in that of the cow and goat.

Specific Gravity. — The specific gravity may be taken by the specific gravity bottle or by a hydrometer. Before using either, the milk should be well shaken and air-bubbles removed.

With a view to the detection of adulteration by water, a special hydrometer is used, which is known as Quevenne's lacto-densimeter. It is furnished with a scale indicating specific gravities from 1042 to 1014. The highest specific gravity of milk yet observed is 1040 to 1041, and the average specific gravity of milk mixed with 50 per cent. of water is 1014 to 1016. The instrument is graduated for use at 15° C., and when employed at a different temperature, a correction must be made in the specific gravity indicated by it. Tables for this purpose are to be found in Gorup-Besanez's Zoochemie, 3rd edition, p. 468. The quantity of water mixed with a sample of milk may be approximately estimated by the subjoined tables.

The specific gravity of milk, with the cream thoroughly mixed with it by shaking, is first ascertained, and if the result is doubtful, another observation is made after the cream has been removed.

Table for estimating the quality of milk by its specific gravity before the removal of the cream :—

Specific Gravity.					
1033 to 29	=	Pure milk.			
1029 „ 26	=	Milk with	10	per cent.	of water.
1026 „ 23	=	„	20	„	„
1023 „ 20	=	„	30	„	„
1020 „ 17	=	„	40	„	„
1017 „ 14	=	„	50	„	„

Table for estimating the quality of milk from which the cream has been removed, by its specific gravity :—

Specific Gravity.					
1037 to 1033	=	Pure milk.			
1033 „ 1029	=	Milk with	10	per cent.	of water.
1029 „ 1026	=	„	20	„	„
1026 „ 1023	=	„	30	„	„
1023 „ 1020	=	„	40	„	„
1020 „ 1016	=	„	50	„	„

** 179. **Constituents of Milk.**—*Casein.*—Casein closely resembles alkali-albuminate* in its characters. It is not precipitated by boiling. It is soluble in alkaline solutions, and is precipitated from them by neutralization, but this precipitation is prevented by the presence of alkaline phosphates. It dissolves in excess of hydrochloric acid, and also, but not so readily, in acetic acid. Milk does not coagulate when it is boiled in a test-tube, but if it is boiled in an evaporating basin, the casein near the surface becomes somewhat dried and forms a scum on the surface; and if this is removed another appears. When milk stands in a warm place, it becomes sour and curdles. If common salt is added to fresh milk, it becomes sour on standing, but does not curdle, for the albumin, separated from the casein by the acid, is kept in solution by the neutral salt. If the solution is boiled, the albumin is coagulated.

Mode of Separating Casein.—As alkaline phosphates are contained in milk, it must be not merely neutralized but rendered distinctly acid, in order to precipitate the casein. The precipitation is not complete unless the milk is diluted.

Add a little acetic acid to milk and warm it to 40° C. The casein and the greater part of the fat separates in large flakes. Moisten a plaited filter with water, and filter the milk; put the filtrate aside, wash the coagulum thoroughly with water, and remove the fat by exhausting it with a mixture of alcohol and ether in the apparatus described in App. § 207. Put this solution aside; the remaining coagulum is casein.†

Mode of Separating Albumin.—Boil the filtrate from which the casein has been precipitated. A precipitate of albumin will be produced. Albumin may also be separated by filtering milk through a porous cell or cone by exhausting the air. A clear fluid will pass through which will not be precipitated by acetic acid, showing that no casein is present, but will be precipitated by boiling or by nitric acid. In the acid liquid from which the coagulable albumin has been removed by boiling, a precipitate is produced by Millon's reagent, although none is occasioned by the addition of nitric acid or mercuric chloride.

Milk-Sugar.—Filter the rest of the fluid in which the albumin has been coagulated. Shake it with ether to dissolve out the fat; remove the ether with a pipette, and then evaporate the fluid to a thin syrup. The milk-sugar will crystallize out gradually in rhombic prisms. It differs from glucose in its crystalline form, (the latter generally occurring in warty crumbling masses,) in fermenting less readily, and in being insoluble in absolute alcohol.

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* Casein is usually regarded as identical with alkali-albuminate. The recent researches of Hoppe-Seyler and Lubavin on its digestion in gastric juice, tend to show that it consists of an albuminous, in combination with a non-albuminous, organic body.

† The casein in human milk cannot be readily precipitated by hydrochloric or acetic acids, and in order to obtain it, magnesium sulphate must be added until the casein is precipitated, and the precipitate must be washed with a strong solution of this salt, and then with alcohol and ether.

The Inorganic Salts of milk, are chlorides, sulphates, phosphates and carbonates of the alkaline and earthy bases. They can only be investigated in the ash. For the method of ignition *see* § 214.

Fats.—The fats may be separated by either of the following methods :—1. Evaporate the mixture of alcohol and ether with which the coagulum was exhausted, and the fat remains.

2. Add to 15 or 20 c. c. of milk, 10 c. c. of a moderately strong solution of caustic soda; shake it vigorously with twice or thrice its volume of ether. Remove the layer of ether, and evaporate it in a water bath, and the fat remains. Ether does not remove the fat from fresh milk, as the casein envelopes the globules, and protects the fat from its action; but soda dissolves these envelopes. Ether will remove 90 per cent. of the butter from milk which has become acid by standing.

** 180. **Mode of Estimating the Quantity of Butter Contained in Milk.**—A rough method of doing this, is to measure the cream which separates from it by Chevallier's cremometer. This is a cylindrical vessel, graduated into a hundred parts. The percentage amount of cream is indicated by the number of divisions it occupies when the vessel is filled with milk to the zero point. This method is quite unreliable.

Vogel's Test.—A much more exact method is that devised by Vogel, which depends on the fact that the opacity of milk is due to the globules it contains, and is in proportion to their number.

The apparatus required for this test are—1. A cylindrical bottle, in which to mix the milk and water. It should hold about 200 c. c., and have a mark on the side at the height of 100 c. c. 2. A test-glass, with parallel glass sides, exactly ½ a centimeter apart, and supported vertically on a metal foot. 3. A pipette graduated in fifths of a cubic centimeter.

Application of the Test.—Before applying this test, it must be ascertained by microscopical examination that the milk does not contain starch granules, or any other impurity in suspension which might increase its opacity. Fill the bottle up to the 100 c. c. mark, with clear spring water. Fill the pipette up to zero with milk (App. § 217), and let 3 c.c. run into the bottle. Mix it well with the water, and fill the test-glass with the mixture. Put it in a tolerably dark room, place a stearine candle at a distance of about three feet from it, and look at the candle through the glass. If the contour of the flame can be readily perceived, pour the liquid back into the bottle, add another ½ c. c. of milk to it, shake it, and look at the candle through it again. Repeat this till the outlines of the flame can no longer be recognized. Then add together the different quantities of milk, so as to find the total amount which has been added, and then ascertain by the following table how much butter the milk contains:—

1·0	c. c. milk,	corresponds to	23·43	per cent of	butter.
1·5	"	"	15·46	"	"
2·0	"	"	11·83	"	"
2·5	"	"	9·51	"	"
3·0	"	"	7·96	"	"
3·5	"	"	6·86	"	"
4·0	"	"	6·03	"	"
4·5	"	"	5·38	"	"
5·0	"	"	4·87	"	"
5·5	"	"	4·45	"	"
6·0	"	"	4·09	"	"
6·5	"	"	3·80	"	"
7·0	"	"	3·54	"	"
7·5	"	"	3·32	"	"
8·0	"	"	3·13	"	"
8·5	"	"	2·96	"	"
9·0	"	"	2·80	"	"
9·5	"	"	2·77	"	"
10·0	"	"	2·55	"	"
11	"	"	2·43	"	"
12	"	"	2·16	"	"
13	"	"	2·01	"	"
14	"	"	1·88	"	"
15	"	"	1·78	"	"
16	"	"	1·68	"	"
17	"	"	1·60	"	"
18	"	"	1·52	"	"
19	"	"	1·45	"	"
20	"	"	1·39	"	"
22	"	"	1·28	"	"
24	"	"	1·19	"	"
26	"	"	1·12	"	"
28	"	"	1·06	"	"
30	"	"	1·00	"	"
35	"	"	0·89	"	"
40	"	"	0·81	"	"
45	"	"	0·74	"	"
50	"	"	0·69	"	"
55	"	"	0·64	"	"

60	c. c. milk,	corresponds to	0·61	per cent of	butter.
70	”	”	0·56	”	”
80	”	”	0·52	”	”
90	”	”	0·48	”	”
100	”	”	0·46	”	”

If cream is to be tested, only one cubic centimeter is to be added at first, and a hlf c. c. at a time afterwards.

Vogel found that about 6 c. c. of pure cow's milk, or 3·7 of cream, added to 100 c. c. of water, were sufficient to form a mixture which quite obscured a candle flame. When 8 c. c. are required, the milk contains about 30 per cent. more water than it ought to do, either from the addition of water, or of creamed milk. When 12 c. c. are necessary, the milk contains 50 per cent. too much water.

THE URINE.

** 181. **Characters of Urine.**—The healthy urine of man is a clear liquid of a golden colour, possessed of a characteristic odour, and having a specific gravity which generally varies from 1018 to 1023, although it may sink as low as 1005, or rise, under opposite circumstances, as high as 1030.

The reaction of the mixed urine of man under normal circumstances is acid. By the term *mixed urine,* we understand a mixture of the different portions of urine passed during twenty-four hours.

When urine is allowed to stand for some hours, it deposits a slight cloudy sediment, which is called *the mucous cloud,* and which consists of mucus holding in suspension a few epithelial cells, derived from the genito-urinary passages. It is usually affirmed that the urine, on exposure to the air, in clean vessels, becomes, after some hours, much more acid than it was when passed. To this change the name of the *acid fermentation* has been given. There are no facts which prove the constant occurrence of this acid change. When the urine is, however, placed for periods which vary very greatly, in open vessels, exposed to air, it ultimately invariably undergoes the so-called *alkaline fermentation, i.e.,* its reaction becomes exceedingly alkaline, it emits an ammoniacal odour, and it becomes turbid, in consequence of the precipitation of phosphate of magnesium and ammonium, of phosphate of calcium, and urate of ammonium.

The acid reaction of healthy human urine is probably due, in great part, to free carbonic acid, to uric and to hippuric acids; it has been commonly believed, however, that acid phosphate of sodium exists in urine and that the acidity of the fluid is chiefly due to its presence.

The alkaline reaction of urine which has become decomposed is undoubtedly due to carbonate of ammonium. Under the influence of putrescent animal substances it may be observed that perfectly fresh urine becomes, in the course of an hour or two, intensely fœtid. Under these circumstances, the urea contained in urine combines with the elements of water and is transformed into ammonium carbonate $C\ H_4\ N_2\ O + H_2O = (N\ H_4)_2\ C\ O_3$. The following experiments throw much light on the proximate causes of the alkaline fermentation of urine:—

Collect 200 cubic centimeters of perfectly fresh urine in a vessel which has been carefully washed with dilute sulphuric acid, and afterwards with distilled water. Examine the reaction of the fluid, which will be found acid, then divide it in four equal parts: 1st, Pour fifty cubic centimeters into a clean beaker, and set it aside to serve as a standard with which to compare the other portions.

2nd, Place fifty cubic centimeters in a clean beaker, and add to it a few drops of urine which has been allowed to become fœtid. After twenty-four hours compare this sample with the first, determining the following points: *a, smell,* which will have become ammoniacal in the second, unchanged in the first; *b, clearness.* The second sample will have become opalescent, or a considerable deposit will have fallen; *c, reaction* will be strongly alkaline in the second, and still acid in the first. The alkaline reaction may be shown to be due to the presence of a volatile alkali by heating the test-paper which has been used, and observing that the reaction which indicated alkalinity disappears on the application of heat; thus the blue colour produced when reddened litmus paper was plunged into the fluid, will disappear, and again give place to red when the paper is heated.

3rd, A third quantity of fifty cubic centimeters is placed in a Florence flask and boiled briskly for some time, then a plug of clean cotton wool is inserted into the neck of the flask whose contents are still boiling, and is thrust down by means of a glass rod. The urine is allowed to boil for some minutes after the insertion of the plug, the flask is then allowed to cool, set aside for many weeks and then examined.

The flask containing boiled urine and protected by the plug of cotton wool, will, if the operator have been sufficiently expert, retain its transparency and its acidity, and when examined with the microscope will present no animal or vegetable forms. On, however, exposing the contents of the flask to the air, the alkaline fermentation will soon occur.

182. Enumeration of the normal constituents of the Urine.

The normal urine of man consists *chiefly* of a watery solution of urea and common salt, mixed with smaller though important quantities of other substances, viz., hippuric acid, creatinine, uric acid, colouring matters yet not accurately investigated, indican, traces of fat, besides ammonium and potassium chlorides, sulphates of potassium and sodium, phosphates of calcium and magnesium, acid phosphate of sodium, silicic acid and iron. To the list of organic substances present in urine, we may add unknown substances which contain sulphur and phosphorus in an unoxidized condition, besides well-known bodies which are certainly present in the urine in certain cases of disease, but which cannot *positively* be classed among the normal constituents.

The abnormal urine of man may contain albumin, grape-sugar, lactic acid and lactates (?), bile colouring matter and bile acids, blood serum and blood cells, hæmoglobin, pus serum and pus cells, carbonate of ammonium, sulphuretted hydrogen, oxalate of lime, xanthine, hypoxanthine, leucine, tyrosine and inosite.

The urine may contain, in addition to the substances which have been previously named, others which have been introduced into the body as drugs or poisons, and which, being excreted by the kidneys, find their way into the urine ; this is the case with many, although probably not with all the metallic salts, with most alkaloids, and with organic bodies of different constitution, as carbolic acid, alcohol, and various vegetable colouring matters.

183. **Urinary deposits.**—Owing to deficiency in the quantity of the urinary water, excess in the quantity of normal ingredients, or presence of substances which are not normally present, we are apt to have urinary sediments or deposits, some of which are composed of structural elements, not usually present, others of the normal or abnormal proximate principles. Amongst such sediments we find most frequently uric acid, urates, ammoniaco-magnesian phosphate, calcium phosphate, calcium oxalate, blood corpuscles, mucus, epithelium, pus, &c.,

** 184. Reactions of Urine treated with some common reagents.

Before commencing a systematic account of the mode of separating the chief constituents of urine, the student may with advantage study the action on this fluid of a few of the common reagents which indicate the presence of the chief ingredients contained. Put about 15 cubic centimeters of urine into a series of test tubes, and try the following experiments :—

1. Add about 5 cubic centimeters of strong nitric acid. No precipitate will occur, either immediately or after standing for some time. The colour of the urine will, however, become darker.

2. To a portion of fresh urine in a test tube add an equal volume of liquor potassæ. After some time a transparent flaky

precipitate will be observed, which separates on boiling, leaving the supernatant fluid of its original colour.

By other experiments it may be shown that solutions of ammonia and caustic soda likewise induce this precipitate, which consists of *earthy phosphates.*

3. Add to 15 cubic centimeters of the urine, about 5 c.c. of a solution of silver nitrate (1-10); an abundant curdy precipitate will fall. This consists of chloride of silver and phosphate of silver; on adding nitric acid to the mixture, the phosphate of silver is dissolved, leaving a quantity of perfectly white chloride of silver, which, after the test tube has been shaken for some time, sinks to the bottom, leaving a clear supernatant fluid.

4. To 15 cubic centimeters of urine which have been strongly acidulated with nitric or hydrochloric acid, add two or three c.c. of a solution of barium chloride. A precipitate of barium sulphate will fall.

5. Pour a strongly acid solution of ammonium molybdate into a test tube, add a few drops of urine and boil; the fluid will become yellow, and a canary-yellow precipitate will fall, composed of phospho-molybdate of ammonium; this indicates the presence of phosphoric acid.

6. To 15 cubic centimeters of urine, in a test tube, add an equal quantity of a solution of caustic baryta. An abundant precipitate will fall, composed chiefly of barium sulphate and phosphate.

7. To the same quantity of urine add about one-third of its volume of a solution of acetate of lead. A white precipitate, consisting of chloride, sulphate and phosphate of lead, will fall; and it will be observed that the urine is to a great extent decolorized.

ON THE METHODS OF SEPARATING, AND ON THE REACTION OF THE PRINCIPAL ORGANIC CONSTITUENTS OF URINE.

** 185. 1. **Preparation of Urea ($C\ H_4\ N_2\ O$) from Urine.**—Take 100 cubic centimeters of urine, and add to it 50 cubic centimeters of a solution made by mixing one volume of a saturated solution of nitrate of barium, with two volumes of a saturated solution of caustic baryta.

A precipitate will form, which is chiefly composed of phosphate and sulphate of barium. On filtering, a clear fluid is obtained which is evaporated to dryness on a water bath. The residue is treated with hot spirits of wine, and the alcoholic solution is likewise evaporated to dryness. On now adding absolute alcohol to the residue the urea is separated, and is obtained from the solution by evaporation. To purify it farther from traces of other organic and saline matters, the crystals of urea must be collected on blotting-paper, strongly pressed between folds of filtering paper, dried on a porous tile, and, if necessary, again dissolved in spirit and recrystallized.

Although urea can be readily obtained from urine, it is more convenient to make use of artificial urea in the experiments which are required to demonstrate its characteristic properties.

As it is altogether beyond the province of this book to refer to matters which concern pure chemistry, it may be merely stated that the artificial urea, which can now be readily purchased, is prepared by mixing, in certain proportions, aqueous solutions of potassium cyanate and ammonium sulphate, evaporating to dryness and extracting the residue with alcohol. During the process ammonium cyanate is first formed, and subsequently this is transformed into its isomer, urea. In order to determine the chief reactions of urea, perform the following experiments :—

1. Take a crystal of urea, and placing it in a water-glass add a few drops of distilled water. It will dissolve with great readiness. Take a couple of drops of the solution and allow it to crystallize on a glass slide, which may be gently heated. A residue is obtained which presents to the naked eye a crystalline appearance, and which under the microscope is seen to be formed of transparent four-sided prisms, terminated by one or two oblique facets (fig. 322).

2. Place a fragment of urea on the tongue, and observe that it possesses a cool, nitre-like taste.

3. Heat a fragment of urea on a piece of platinum foil, or on a platinum spatula, over a gas or spirit-lamp. The urea will first melt, then solidify, and ultimately burn away rapidly without leaving a trace of ash or unburned carbon.

4. Place a tiny crystal of urea on a glass slide; dissolve it in distilled water, and then add a drop of strong and perfectly colourless nitric acid. Crystals will form which consist of a compound of nitric acid and urea ($C H_4 N_2 O, H N O_3$). These are much less soluble than crystals of urea. Nitrate of urea is comparatively insoluble in dilute nitric acid. Nitrate of urea crystallizes generally in the form of six-sided tables (fig. 323).

From highly concentrated urine of man, large quantities of nitrate of urea may be sometimes obtained, without any previous evaporation, by merely adding pure nitric acid. In any case, however, nitrate of urea may be obtained in a crystalline form by evaporating urine nearly to a syrupy consistence, decanting the liquid from the salts which have separated out, and then adding an equal volume of pure nitric acid.

5. Perform an experiment similar to the preceding one, substituting a solution of oxalic acid for the nitric acid. A crystallization of oxalate of urea ($C H_4N_2O,C_2H_2O_4$) is obtained (fig. 324).

6. Take one cubic centimeter of a solution of pure urea (containing 5 grammes dissolved in 100 grammes of distilled water). Then add cautiously a solution of mercuric nitrate; a curdy white precipitate forms, which consists of combinations of urea with mercuric oxide. On adding a drop of the mixture of urea and mercuric nitrate to a drop of a cold saturated solution of sodic carbonate no reaction will be observed until an excess of the mercuric salt has been added. Then there is produced a very characteristic yellow colour, due to the precipitation of mercuric hydrate. On this reaction is based Liebig's method for the determination of urea.

7. Place one cubic centimeter of a solution such as that used in the last experiment, in a test tube, and then fill the latter exactly with a solution of sodium hypochlorite. Invert the tube once or twice, and plunge it into a basin containing mercury. A most vigorous evolution of gas takes place; this consists of nitrogen.

The reaction which occurs is illustrated by the following equation:—

$$C\,H_4N_2O + 3\,NaCl\,O = 3\,NaCl + CO_2 + 2\,H_2O + 2\,N.$$

The carbonic acid which is generated in the reaction is absorbed by the solution of sodium hypochlorite.

Instead of sodium hypochlorite, the similar salt of *potassium* or *calcium* might be used in this experiment.

** 186. **Separation of uric acid ($C_5\,H_4\,N_4\,O_3$) from Urine.**—Place 200 cubic centimeters of urine in a narrow glass cylinder, and add two or three cubic centimeters of pure nitric acid. After twenty-four hours a brick coloured or brown sediment will have subsided, which consists of crystals of uric acid, strongly tinted with the colouring matter of urine. These present, under the microscope, the most various forms, the more common being rhombic tables or columns and lozenge-shaped crystals; the yellow or brown colour which such crystals possess is very characteristic of uric acid.

Decant the urine from the red sediment of uric acid, which may be freely washed with distilled water, as uric acid requires 14,000 times its weight of cold and 1,800 times its weight of hot water to dissolve it. The sediment may then be collected on filtering paper and subjected to the following tests:—

1. Place a small quantity of the crystals on a microscopic slide, and add a drop of liquor potassæ. The crystals dissolve, and a solution of urate of potassium is obtained ($C_5\,H_2\,K_2\,N_4\,O_3$).

Now add carefully an excess of nitric or hydrochloric acid, when uric acid will be again obtained in the form of crystals, which may be further examined.

It may be well to state that uric acid often occurs as a deposit in urine which has not been artificially acidified, and that the crystallographic characters of the substance are very various and sometimes puzzling. The typical crystals of uric acid are undoubtedly rhombic plates with extremely obtuse angles; the typical form is, however, very frequently modified; thus, spindle-shaped figures are formed by the rounding of the obtuse angles, or the primary form is so modified that needles are formed which occur in groups (fig. 305). Not at all unfrequently we have the primary form so modified that the crystals resemble hexagonal plates. Experience gained by a frequent comparison with accurate drawings of the various forms of crystals of uric acid, can alone enable the observer rapidly to identify uric acid. When any doubts exist as to the identity, it is well to dissolve the suspected crystals in liquor potassæ, and to proceed as directed above, for by neutralizing an alkaline urate with acid, some of the commoner, and therefore easily identified shapes of uric acid crystals, are obtained.

2. Place a very small quantity of the reddish crystalline deposit in a watch glass; add four or five drops of nitric acid and heat very cautiously over a small spirit-lamp flame. The uric acid will dissolve, and on evaporating to dryness, a reddish-yellow residue is obtained. On exposing this residue to the vapour of ammonia, or adding, by means of a thin glass rod, a small quantity of solution of ammonia, a beautiful purple red colour is developed, which, on the subsequent addition of a little solution of caustic potash, assumes a violet tint. This reaction has received the name of the *Murexide* Test.

* 187. **Separation of Hippuric Acid** ($C_9\ H_9\ NO_3$).— After urea, hippuric acid is the organic compound present in largest quantity in the urine of man, the mean quantity excreted per diem amounting at least to one gramme. The difficulties attending the separation of hippuric acid from the urine of man are, however, great, and it is therefore advisable that the student should learn to isolate this substance when it is present in larger quantities than normal in the urine. As the urine of herbivora contains large quantities of hippuric acid, it may be advantageous to use for the experiment to be described cows' or horses' urine, or the urine of men in whom an excessive excretion of hippuric acid has been induced; this may be done by administering to a man ten or fifteen grammes of benzoic acid ten or twelve hours before the urine is collected.

It is a fact worthy of remembrance that when benzoic acid is administered to healthy men, large quantities of hippuric (glyco-benzoic) acid are excreted. There appears to be always in the system a quantity of glycocine ($C_2\ H_3\ (N\ H_2)\ O_2$), which although it is never excreted as such, is capable of being seized upon by the radical of benzoic acid, so as to yield hippuric acid. By comparing the formulæ of glycocine and hippuric acid, exhibited below, it will be seen that the latter can be represented as derived from the former by the substitution of ($C_7\ H_5\ O$) for H, thus:—

Glycocine . . . . . . $C_2 H_3 (N H_2) O_2$
Hippuric acid . . . . $C_2 H_2 (N H_2) (C_7 H_5 O) O_2$.

Take 200 cubic centimeters of the fresh urine of the cow and concentrate it, by heating on the water bath, to forty cubic centimeters. Then add hydrochloric acid, and set aside until next day. A large quantity of hippuric acid will have separated in the form of a brown crystalline mass. Wash with cold water, press the crystalline mass between folds of filtering paper; dissolve in as little boiling water as possible, add a little pure animal charcoal (*i.e.*, animal charcoal which has been in contact with dilute hydrochloric acid for many days, and then thoroughly washed with water), and filter. The filtrate should be concentrated and allowed to crystallize. (For other methods of separating hippuric acid, especially when existing in small quantities, the reader is referred to Hoppe-Seyler's "Handbuch der physiologisch-und pathologisch-chemischen Analyse, 1870, p. 157.)

Having obtained nearly pure hippuric acid, the following experiments may be tried.

1. Dissolve a fragment in boiling water, and allow a drop of the solution to crystallize on a microscope slide. The acid usually separates in the form of transparent prisms which are single, or occur in radiating groups, and generally present four sides parallel to their long axis; their ends are terminated by two or four planes. Their primary form is a right rhombic prism (fig. 313).

2. Heat a fragment of hippuric acid in a small glass tube, with a little soda-lime; the ammonia which is given off, and which can readily be detected by its odour, proves that the body under examination contains nitrogen.

3. Mix a fragment of hippuric acid with strong nitric acid in a small porcelain crucible. Boil and then evaporate to dryness; on heating the residue, a very characteristic odour of nitro-benzol is developed.

* 188. **Separation of Creatinine** ($C_4 H_7 N_3 O$) **from Urine.**—To 300 cubic centimeters of urine add milk of lime until the reaction of the fluid is decidedly alkaline. Then add a solution of chloride of calcium as long as a precipitate falls. After the precipitate has been allowed partially to subside, filter, evaporate the filtrate to dryness in a basin or the water-bath, and add to the yet warm residue thirty or forty cubic centimeters of 95 per cent. alchohol. Stir and decant the contents of the basin into a beaker, taking care to add the alcoholic washings of the basin. Set aside the beaker in a cool place. Filter and wash the insoluble residue with a little more spirit. If the filtrate and washings amount to more than 50 c. c., concentrate at a gentle heat to that volume. Allow the fluid to cool, and then add half a cubic centimeter of an

alcoholic solution of chloride of zinc, absolutely free from the least trace of acid, and stir for some time. Set the beaker aside for three or four days in a cellar. At the end of that time the whole of the creatinine will have separated in combination with zinc chloride. It should be collected on a filter and washed with pure spirit; the substance left on the filter consists of chemically pure chloride of zinc-creatinine ($(C_4 H_7 N_3 O)_2, Zn Cl_2$). This most characteristic compound is very slightly soluble in cold water and insoluble in cold alcohol; it crystallizes from urine in the form of bundles of needles.

From chloride of zinc-creatinine, the pure substance is obtained by boiling with freshly prepared and thoroughly washed hydrate oxide of lead for half an hour or longer. On filtering the fluid, and evaporating to dryness, creatinine is obtained, which may be dissolved in alcohol and crystallized.

Creatinine is very soluble in cold alcohol. The following experiments may be performed with it:—

1. When a few drops of a solution are allowed to evaporate spontaneously, colourless prisms are obtained (fig. 302).
2. The taste of the solution is strongly alkaline.
3. The reaction to test-paper is intensely alkaline.
4. A concentrated solution of chloride of zinc added to creatinine, causes the immediate precipitation of the zinc compound, which is always crystalline.

** 189. **Separation of the colouring matters of Urine.**—Under various names, among others, that of Urohæmatine, different writers have described the substance, or mixture of substances, which they considered to be the cause of the colour of healthy urine (Scherer, Harley, Heller). We are now perfectly convinced that no one colouring matter, capable of accounting for the normal golden or amber color of human urine, has been separated.

The following experiments may be performed, as they throw some light on the reactions of the normal urinary colouring matter:—

1. Take 200 cubic centimeters of urine and precipitate with neutral acetate of lead; an abundant precipitate falls, which consists of lead salts of acids present in the urine and which contains a portion of the urinary colouring matter. Filter, and observe that the filtrate from this precipitate is not altogether colourless. Add to the filtrate basic acetate of lead, when a further precipitate will form, which, when separated, leaves a colourless filtrate.

Now unite the precipitates caused by neutral and basic acetates of lead, and treat the mixture with alcohol acidulated with hydrochloric acid. A red fluid will be obtained, which, on filtration and evaporation, yields a reddish-black residue, insoluble in water.

That this is not, as was supposed, the colouring matter of urine, is now admitted. The researches of Dr. Harley, although failing to discover any one normal urinary colouring matter, show that the so-called urohæmatine contains a mixture of several pigmentary substances.

2. Passing from urohæmatine, the student's attention is to be drawn to the constant presence in urine of a very well-defined body—viz., indican, or white indigo ($C_{16}\ H_{12}\ N_2\ O_2$)—which may readily be converted into indigo-blue and indigo-red. To the indican present in urine, Heller, who first discovered its presence, without, however, being aware of its nature, gave the name of Uroxanthine, and to the indigo-blue and indigo-red obtained from it, the names of Uroglaucine and Urrhodin respectively.

For the method of obtaining indican, the reader is referred to Hoppe-Seyler (Op. Cit. p. 163); it will be sufficient if the student performs the following experiments:—

Precipitate 100 cubic centimeters of perfectly fresh urine with acetate of lead. The fluid is filtered. The filtrate contains the whole of the indican. A strong solution of ammonia is added, which precipitates hydrated lead oxide, together with indican. The precipitate is collected on a filter, washed with water and dilute hydrochloric acid. Very often the filter is seen to contain blue particles, in consequence of the production of indigo-blue, which contrasts with the chloride of lead with which it is mixed.

The filtrate, when left to itself for twenty-four hours, generally becomes covered with a bluish purple film, consisting of indigo.

3. Several hundred cubic centimeters of pure urine are precipitated by acetate of lead and then filtered; the filtrate is treated with excess of sulphuretted hydrogen, boiled and filtered; the filtrate is now poured into an equal volume of pure and strong hydrochloric acid. The fluid becomes either violet or indigo-blue; it is allowed to stand for twelve hours, and diluted with an equal volume of water. After about twenty-four hours, a deposit will generally have formed, which is collected on a filter, washed, and dried. When treated with ether, the deposit will generally yield to it a red colouring matter, whilst indigo is left behind, and is to be purified by solution in boiling alcohol.

The student will remember that indigo-blue only differs from indican in the possession of two additional atoms of hydrogen,—

Indican, or white indigo	$C_{16}\ H_{12}\ N_2\ O_2$.
Indigotin, or blue indigo	$C_{16}\ H_{10}\ N_2\ O_2$.

In the production of indigo-blue from indican there are other substances formed, such as a form of sugar, which is an isomer of glucose, but unfermentable, and the imperfectly investigated body, indigo-red, which has already been alluded to.*

The following reactions may be tried with indigo-blue :—

(*a.*) Shake a fragment of indigo-blue with ether; the substance is found to be very scantily soluble. Ether, however, dissolves enough to acquire a faint blue tint.

(*b.*) Place a fragment in a narrow glass tube and heat; it will sublime and be deposited in the cool part of the tube. If the latter be very narrow and thin, it may be examined microscopically. The sublimate of indigo is then seen to consist of microscopic needles and plates.

METHODS FOR THE QUANTITATIVE ANALYSIS OF URINE.

**** 190. Determination of the total quantity of Urine passed in a given time.**—Before describing briefly the methods which are employed for the determination of the more important urinary constituents, attention must be drawn to the fact that, as a general rule, quantitative analysis of urine throws little or no light on the rate and character of the tissue changes going on in the animal body, unless the analysis be made of a specimen of urine which represents the average excretion of a known period, during which the conditions of the animal have been ascertained as accurately as possible.

These remarks will be better understood when it is stated that we can obtain the most valuable information relating to the urinary secretion if we collect, mix, and then measure the whole of the urine passed in twenty-four hours. Having ascertained the total volume of urine passed in twenty-four hours, two hundred cubic centimeters will suffice for the great majority of quantitative analyses.

The urine of man must be collected in perfectly clean glass vessels which, in accurate experiments, should, before being used, be washed with dilute sulphuric acid, and then with water. The collecting-vessel may be graduated or not; in the latter case, the urine is carefully poured, if necessary, in successive portions, after being mixed, into a cylinder capable of holding a litre of water, and divided into 200 parts; so that each division indicates 5 cubic centimeters.

It is frequently of use to collect the urine of dogs and rabbits, especially when experiments are made on the physiological action of drugs.

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* In many cases of disease, urine contains so much indican, that the following reaction may be observed :—

To five cubic centimeters of fuming hydrochloric acid, add from one to two cubic centimeters of urine. A violet colour is produced, which passes into red.

In these cases, cages are employed, whose walls are made partly of sheet iron or zinc, and partly of wire netting. The floor of the cage should be made of thick glass rods (about four-tenths of an inch in diameter), placed very closely together. These rods are so arranged that the spaces between them will allow urine to trickle away, whilst the solid excreta are retained.

The glass rods are firmly inserted into the wooden base of the cage; this is furnished with a drawer, into which is accurately fitted a flat glass or porcelain dish, such as is used by photographers in washing photographs. The dish is perforated by a hole, in which a tube (preferably of glass) is accurately fitted, and leads to the collecting vessels outside.

If care be taken to wash the glass-rod bottom of the cage and the collecting-glass dish placed beneath it, the urine may be collected in a state of great purity.

** 191. **Determination of the specific gravity of Urine.**—This may be effected in either of the two ways described in App. § 216, for the determination of the specific gravity of fluids, viz., by means of a hydrometer or with the specific gravity bottle.

The hydrometer employed for taking the specific gravity of urine is called a urinometer; in this country its stem is usually divided so as to indicate densities ranging from 1,000 to 1,060 (water being 1,000); it is preferable to use two urinometers: one indicating densities from 1,000 to 1,030, the other from 1,030 to 1,060. The length of the stem being the same as that of the ordinary instruments, the accuracy of the reading will be much increased. Before using a urinometer, its accuracy should be checked by immersing it in fluids of known specific gravity. If the specific gravity of three samples of urine be accurately taken with the bottle, data are obtained for checking the accuracy of the urinometer.

Although, under certain circumstances, important information may be obtained by a determination of the specific gravity of an isolated sample of urine, generally it is only when the specific gravity of a sample of the mixed and measured urine of the twenty-four hours is ascertained, that we learn much from the experiment.

A knowledge of the specific gravity enables one to form a near approximation to the total quantity of solid matter excreted by the kidneys in a given time.

It has been empirically determined that the specific gravity of urine generally bears a close relation to the solid matters which it contains in solution. Sir Robert Christison pointed out, many years ago, that if the whole numbers which express the difference between the density of a sample of urine and the density of water (expressed as 1000) be multiplied by the factor 2·33, the product represents very closely the weight of the total solids contained in 1,000 parts, by weight, of urine. Subsequent observers have determined that whilst Dr. Christison's formula yields very correct results when applied to urines of specific gravities above 1018, for urines of lower specific gravity greater accuracy is obtained by substituting the factor 2 for 2·33.

The following example will suffice to show the method of calculating approximately the total solid matter excreted in the urine in twenty-four hours:—

A man passes in twenty-four hours 1,575 cubic centimeters of urine of specific gravity 1023, and it is desired to obtain an approximate estimate of the total urinary solids.

1st. We find the total solids (expressed in any particular units of weight) contained in 1,000 parts (expressed in the same units of weight) by Dr. Christison's formula, thus, if the unit be the gramme, and the quantity of solid matter in 1,000 grammes be represented by x,

$$x = (1023 - 1000)\ 2{\cdot}33 = 53{\cdot}59.$$

2nd. We require to know the weight of the whole of urine. As its density is 1,023, and the quantity 1,575 cubic centimeters, the weight in grammes is at once found by the following proportion:—

$$1000 : 1023 :: 1575 : x$$

$$x = \frac{1023 \times 1575}{1000} = 1611.$$

3rd. Knowing the weight in grammes of the urine of twenty-four hours, and the approximate weight of total solid matters in 1,000 parts, by weight, of urine, we obtain the total solids passed in twenty-four hours expressed in grammes:—

$$1000 : 53{\cdot}59 :: 1611 : x$$

$$x = 86{\cdot}33 \text{ grammes.}$$

It is to be noted that the result obtained by such calculations is merely an approximation to the actual number which would be ascertained by the direct method, to be immediately described; the approximation is, however, sufficiently close to be useful.

192. **Determination of the Total Solid Matters contained in Urine.**—If we know the total volume of urine passed in twenty-four hours, and it be desired to ascertain, by direct weighing, the total quantity of solid matter contained in it, 10 or 15 cubic centimeters of the mixed urine are poured from a very accurately graduated pipette into a weighed porcelain or glass capsule, which is heated over the water bath, or in the water oven (fig. 339),

until a nearly dry residue is obtained. The capsule with its contents is then heated in an air oven whose temperature is maintained at 120° C. The capsule is, after some time, allowed to cool in an exsiccator (fig. 340) and rapidly weighed. The drying and weighing should be repeated until the weight of the capsule and residue is constant. In order to insure accuracy, the capsule in which the evaporation is carried on should be fitted with a ground glass plate, which should be placed over it, when it is transferred from the air oven to the exsiccator, and from the exsiccator to the balance.

It is absolutely essential that the weighing should be conducted with the greatest possible rapidity, as the dried urinary solids are highly hygroscopic.

Instead of measuring the urine used in the analysis, a weighed quantity may be taken.

** 193. Determination of the Amount of Chlorine contained in Urine.

By Liebig's Method.—It has been already mentioned that when a solution of mercuric nitrate is added to a solution of urea, a dense white precipitate is formed, which consists of compounds of urea with mercuric oxide.

If the solution of mercuric nitrate be sufficiently diluted, and be added in sufficient quantity, the compound formed contains four molecules of mercuric oxide for each molecule of urea.

If, however, a solution of mercuric nitrate be added to a solution of urea and chloride of sodium, no precipitate will at first be formed, the reaction between the urea and oxide of mercury not occurring until the double decomposition between the mercuric nitrate and sodium chloride has been completed, thus:—

$$\mathrm{Hg\,2N\,O_3 + 2\,Na\,Cl = Hg\,Cl_2 + 2\,Na\,N\,O_3}.$$

As soon, however, as this has occurred, a white precipitate of the mercuric oxide and urea compound falls.

Liebig's method of determining the amount of chlorine in urine is based upon the reactions which have been referred to.

In order to enable the student to determine the amount of chlorine by Liebig's method, we shall describe, in the first place, the method of preparing the standard solution of nitrate of mercury, and, in the second place, the method to be followed in determining by its aid the quantity of chlorine in urine.

Preparation of standard solution of mercuric nitrate for the estimation of chlorine in Urine.

The following solutions are required:—

1st. A solution of mercuric nitrate of such a strength that one cubic centimeter shall correspond to 10 milligrammes (0·010 grm.) of sodium chloride.

This solution may be made by dissolving twenty grammes of perfectly pure metallic mercury in boiling nitric acid, until a drop of the acid fluid does not cause a precipitate when added to a solution of common salt. The acid fluid is concentrated by heating over a water bath until it is of syrupy consistence. It is then diluted with nearly a litre of distilled water.

Unless a great excess of nitric acid has remained after the evaporation, a white precipitate, consisting of a basic nitrate of mercury, will fall, and must be separated by filtration. Before performing the latter operation, a few drops of nitric acid may, however, be added, as they will cause the re-solution of a considerable part of the precipitate, without rendering the liquid too acid. The solution of mercuric nitrate thus made must be set aside until the other reagents which are required for determining its strength are prepared.

2nd. A solution made by dissolving in distilled water 20 grammes of pure sodium cloride and diluting to one litre. The salt is fused before being weighed.

Ten cubic centimeters of this solution contain 0·200 grm. of NaCl.

3rd. A solution made by dissolving 4 grammes of pure urea in distilled water and diluting to 100 c. c.

4th. A solution of sodium sulphate, saturated at ordinary temperatures.

In order to determine the strength of the solution of mercuric nitrate, it is poured into a burette (preferably a Mohr's burette, with glass stopcock) of a capacity of 50 cubic centimeters, and divided into 10ths of a cubic centimeter.

Ten cubic centimeters of the standard solution of chloride of sodium are then measured by means of a pipette, and poured into a glass beaker.

To this is added 3 cubic centimeters of the solution of urea, and 5 cubic centimeters of the solution of sulphate of sodium. The solution of nitrate of mercury is now allowed to flow gently into the beaker; as the drops fall into the fluid contained in the latter, a white precipitate is seen to form, which, however, dissolves at once, or when the fluid is stirred. On adding more of the solution of nitrate of mercury, the fluid becomes opalescent but no precipitate occurs until the reaction is completed, *i.e.*, until the whole of the chloride of sodium has been decomposed.

The number of cubic centimeters of the solution of mercuric nitrate which has been added is read off; if, for example, 12·7 cubic centimeters of the solution had to be added in order to induce a permanent precipitate, we conclude that this quantity of solution contains the quantity of mercuric nitrate required to decompose 0·200 gramme of NaCl. As it is convenient to have a solution of which 10 cubic centimeters shall be equivalent to 0·100 grammes of NaCl., we must take our solution and dilute it to the required extent. In the assumed case, 12·7 cubic centimeters contained as much of the mercurial salt as corresponded to 0·200 grammes of NaCl., *i.e.*, as much as would be required in 20 cubic centimeters of solution. If we therefore diluted 12·7 cubic centimeters with 7·3 cubic centimeters of water, we should obtain 20 cubic centimeters of a solution of which 10 cubic centimeters would be exactly capable of decomposing 0·100 gramme of NaCl.

But as in preparing such a standard solution we deal with large quantities of fluid, it is well to effect the dilution of the whole at once.

Thus, let us suppose that we have 800 cubic centimeters of the solution, of which 12·7 cubic centimeters are equivalent to 0·200 gramme of NaCl.

As 12·7 cubic centimeters require the addition of 7·3 cubic centimeters of water, it is easy to find how much 800 cubic centimeters require, viz., 459·8 cubic centimeters. If we then measure out very accurately this quantity of distilled water, and add it to our solution, we obtain 1259·8 cubic centimeters of a solution of which 10 cubic centimeters represent 100 milligrammes of NaCl., or 60·65 milligrammes of Cl.

Having made the standard solution of nitrate of mercury for the estimation of chlorine, we must, before analyzing urine, prepare a solution which we shall designate as *Baryta Mixture.*

This is prepared by mixing two volumes of a solution of barium nitrate, saturated in the cold, with one volume of a solution of caustic baryta (barium hydrate), similarly saturated.

Two volumes of the urine to be analyzed (say 40 cubic centimeters) are now mixed with one volume (say 20 cubic centimeters) of baryta mixture. An abundant precipitate falls, consisting chiefly of a mixture of phosphate, sulphate and carbonate of barium. (This removal of phosphates is essential, as these salts are precipitated by the solution of nitrate of mercury.)

The fluid in which the precipitate has formed is filtered, care being taken that the filter is not moistened.

As the filtrate contains one-third of its volume of baryta mixture, it is convenient to take for analysis 15 cubic centimeters. This quantity will exactly correspond to 10 cubic centimeters of urine. It is convenient, therefore, to have, in addition to pipettes graduated so as to deliver 20 and 40 cubic centimeters, one which delivers exactly 15 cubic centimeters of fluid. The measured portion of filtrate is *very slightly* acidified by adding, drop by drop, exceedingly dilute nitric acid, and then the solution of nitrate of mercury is allowed to flow in, at first rather rapidly, afterwards *guttatim*, until a permanent and dense cloud, not affected by vigorous stirring, makes its appearance.

The number of cubic centimeters used, multiplied by 0·010, indicates the amount of chlorine, in fractions of a gramme, calculated as NaCl., contained in 10 cubic centimeters of urine. Thus, if 8·56 cubic centimeters of the standard solution of chlorine were added, the quantity of Cl., calculated as NaCl., in 10 cubic centimeters, would be 0·085 gramme.

It must be remarked that if a urine contains albumin, this substance must be removed by boiling and filtration before the determination of chlorine by Liebig's method can be effected.

194. *Determination of chlorine by means of nitrate of silver.* —In cases where the quantity of chlorine is exceedingly small, the following method is much to be preferred to that already described.

Ten cubic centimeters of urine are placed in a platinum capsule, together with 2 grammes of pure potassium nitrate (quite free from chlorine), and evaporated to dryness. The residue is ignited at a moderate heat until the whole of the carbon has disappeared.

The crucible is allowed to cool, and the saline mass which it contains is dissolved in distilled water, a little nitric acid being added. The estimation of chlorine may then be effected by those methods which are to be found described in text-books on chemical analysis. The chief of these methods consist (*a*) in precipitating the chlorine as chloride of silver, &c., washing, burning, and weighing the precipitate; and (*b*) in adding to the neutralized solution of the chloride, mixed with a drop of potassium chromate, a standard solution of nitrate of silver. The nitrate of silver causes a white precipitate of chloride of silver, when added to such a solution, until the whole of the chlorine has been precipitated. Then, however, the addition of a single drop more produces a deep salmon-red colour, due to the formation of silver chromate.

**** 195. Determination of the amount of Urea found in Urine.**

I. *By Liebig's Method.*—In order to determine the amount of urea by Liebig's method, we require (*a*) baryta mixture as used in the determination of the amount of Cl. in urine, and (*b*) a standard solution of nitrate of mercury, prepared in the same manner as that used for Cl. determinations, but containing much more mercury. In making this solution, dissolve about 75 grammes of pure mercury in pure nitric acid, adopting all the precautions previously suggested, and dilute to the volume of one litre.

In order to graduate the solution of mercuric nitrate for urea, we must pour into a beaker 10 cubic centimeters of a standard aqueous solution of pure urea, containing 2 grammes of perfectly pure urea in 100 cubic centimeters. The quantity of solution in the beaker will then contain 0·200 gramme of urea.

The solution of mercuric nitrate is then added and the fluid stirred; an abundant snow-white precipitate falls. When the precipitation appears to be nearly completed, a drop of the fluid holding the precipitate in suspension is added to a drop of solution of sodium carbonate on a porcelain slab. If the urea be not completely precipitated, no change of colour will be observed when the two fluids are mixed. The mercuric nitrate solution is then added drop by drop, and the process of testing with the solution of $Na_2 CO_3$ on the slab repeated from time to time. At last a yellow colour will appear. This will indicate that the solution of mercury has been added in excess. The number of cubic centimeters of solution added indicates the number of c. c. which are equivalent to 0·200 gramme of urea. As it is convenient to have a solution of mercuric nitrate, of which 10 cubic centimeters shall precipitate 100 milligrammes of urea (0·100), or 1 cubic centimeter 10 milligrammes, it is essential to dilute the solution which has been prepared, in the same manner as was indicated in the case of the solution for the determination of chlorine.

Having prepared the *solution of mercuric nitrate for urea*, and the *baryta mixture*, the analysis of urine can be rapidly effected. 40 cubic centimeters of urine are mixed with 20 cubic centimeters of baryta mixture; 15 cubic centimeters of the filtrate are precipitated with the mercury solution, until a yellow reaction with solution of $Na_2 CO_3$ is obtained.

The number of cubic centimeters of the mercury solution used, minus 2, and multiplied by 0·010 gramme, indicates very closely the amount of urea, expressed in fractions of a gramme, contained in 10 cubic centimeters of urine, provided that the urine be of average composition, *i.e.*, that it contains no abnormal substances, that the amount of chlorine in it be about the average, and that it be neither very concentrated nor very dilute.

The statements made in the preceding paragraph indicate many circumstances which have to be taken into account, and many corrections which have to be introduced in order to give to Liebig's method the accuracy of which it is capable.

In pointing out these corrections, an explanation must be given of the empirical statement, "*that the number of cubic centimeters of mercury solution used, minus 2, and multiplied by* 0·01 *grm., indicates very closely the amount of urea, expressed in fractions of a* *gramme, contained in* 10 *cubic centimeters of urine*." The reason for subtracting 2 cubic centimeters is, that in average urines this volume of the solution is required to decompose the chlorides, and does not, therefore, take part in the urea reaction.

If this correction be constantly introduced in a series of observations, and, as has been already pointed out, the urine be not of very exceptional composition, results are obtained which are very nearly correct, and which are comparable the one with the other If, however, the urine in cases of pneumonia or of fevers were under investigation, the error introduced by the application of this arbitrary correction would generally be very great.

In such cases we must adopt a more scientific method of avoiding the error introduced by the presence of chlorides. We must in the first place determine, by the *standard solution of mercuric nitrate for chlorine*, the amount of chlorine, calculated as NaCl. present in 10 cubic centimeters of the urine, *i.e.*, in 15 cubic centimeters of the filtrate obtained on mixing two volumes of urine with one volume of baryta mixture, and we must then remove the whole of the Cl. from a fresh quantity of filtrate by a standard solution of nitrate of silver. To do this we require a solution of nitrate of silver exactly equivalent to the solution of nitrate of mercury which has been used. If 11·601 grammes of fused silver nitrate be dissolved in distilled water, and diluted to the volume of 1 litre, the solution will be of the required strength, *i.e.*, 1 cubic centimeter will exactly precipitate 0·010 gramme of chloride of sodium.

Take 30 cubic centimeters of the filtrate from the mixture of baryta mixture and urine, and having added a drop of nitric acid, pour in from a burette, or from a finely divided pipette, twice as many cubic centimeters of the nitrate of silver solution as the number of cubic centimeters of nitrate of mercury solution required in the chlorine determination. A precipitate of chloride of silver will fall, and the filtrate may now be subjected to analysis for urea.

An example will help to make the course of these operations clear.

Forty cubic centimeters of the urine of a boy suffering from typhus fever were mixed with 20 cubic centimeters of baryta mixture, and the fluid was filtered. 15 cubic centimeters of the filtrate were placed in a beaker, and the *standard solution of mercury for chlorine* was added, until a permanent and dense cloud had formed. The number of cubic centimeters added was 4·5. As each cubic centimeter of the standard solution corresponds to 0·010 gramme of Cl. calculated as NaCl, the quantity in 10 cubic centimeters amounted to 0·045 gramme. 30 cubic centimeters of the filtrate from the baryta mixture and urine were now taken and treated with 4·5 × 2, *i.e.*, 9 cubic centimeters of nitrate of silver solution. The fluid was filtered. Now 39 cubic centimeters of the mixture of urine, baryta solution, and silver nitrate solution, contained 20 cubic centimeters of urine. On therefore taking $\frac{39}{2}$ or 19·5 cubic centimeters of the filtrate, after the precipitation of the chloride of silver, we obtained a quantity of fluid which contained all the urea present in 10 cubic centimeters of the original urine.

It may be well to state that when, as in many cases of acute disease, the amount of chlorine present is very small, nearly accurate results are obtained, if no correction for chlorine be introduced.

Other corrections must be introduced into Liebig's method under peculiar circumstances: these will be stated dogmatically, the student being referred to larger books for their explanation.

1st. When, in determining the amount of urea in 15 cubic centimeters of mixture of urine and baryta solution, the number of cubic centimeters of mercury solution added exceeds 30, we must repeat the operation, adding to 15 cubic centimeters of the fluid a quantity of distilled water equal to the difference between 30 and the number required in the first operation.

2nd. When the amount of solution of nitrate of mercury added to 15 cubic centimeters of the filtrate from the mixture of urine and baryta mixture, is less than 30 cubic centimeters, 0·1 cubic centimeter must be subtracted from the amount of mercury solution required, for every 5 cubic centimeters less than 30 cubic centimeters.

This correction is of little importance.

II. *Davy's method for the determination of Urea.*

This excellent method is based upon the fact already mentioned, that when a solution of urea ($CH_4 N_2 O$), such as urine, is treated with a solution of a hypochlorite, it splits up into carbonic acid, water, and nitrogen gas. If the mixture be effected in a long, graduated tube, and this be inverted and placed over mercury, the whole of the N accumulates on the surface of the fluid, the carbonic acid being absorbed by the solution of hypochlorite used. From the volume of N evolved the quantity of urea present may be calculated. (For details of this method the reader is referred to a Treatise on the Pathology of the Urine, by Dr. Thudichum, London: Churchill, 1858.)

Davy's process is, like Liebig's, not absolutely correct. Uric acid, and other nitrogenous substances present in urine are decomposed by hypochlorites; as their quantity is, however, comparatively very small, the error introduced is not large. The writer can vouch, from personal observations, of the great accuracy of this method when applied to solutions of pure urea, and believes that, if carried out with the apparatus devised by Dr.

Hüfner for the determination of urea by solutions of alkaline hypobromites, it would prove the most useful and reliable method for the determination of urea.

* 196. **Determination of the Amount of Uric Acid in Urine.**—Uric acid is usually determined by precipitation with dilute nitric or hydrochloric acid, the crystalline precipitate being washed, dried, and weighed.

Take 200 c. c. of the urine and add to it 5 c. c. of dilute hydrochloric acid of density 1·11. Set aside in a cellar for 24 hours. Collect the uric acid on a weighed filter, and wash thoroughly with distilled water. Dry the filter and uric acid in a water oven at a temperature of 100° C. Allow the dried filter to cool under an exsiccator (in watch glasses, &c.,) and weigh. The weight of the filter and uric acid, minus the weight of the filter paper, gives the amount of uric acid precipitated. To this must, however, be added the quantity of uric acid which has been held in solution by the urine and hydrochloric acid, and by the washings of the filter. The whole of these fluids are therefore mixed and measured, and for every 100 c. c., 0·0038 grammes of uric acid must be calculated (Neubauer). The number thus calculated, added to that of the uric acid collected on a filter, gives the amount of uric acid contained in the urine. The number is, however, only an approximation to the truth.*

** 197. **Determination of the Amount of Phosphoric Acid contained in Urine.**—The Phosphoric acid contained in urine exists partly in a state of combination with the alkaline earths, magnesia, and lime, but chiefly in combination with alkalies. If we render the urine alkaline by the addition of ammonia, the former are precipitated, leaving the alkaline phosphates in solution. It is customary to state the amount of phosphoric anhydride corresponding to the phosphoric acid in the urine. In determining the quantity of phosphoric acid in urine, we may merely determine the total quantity existing in the fluid, or we may determine the total quantity first, and then the quantity which remains after the precipitation of the earthy phosphates.

The volumetric method for the determination of phosphoric acid in urine is based upon the following reactions:—

(*a*) When a solution of a phosphate acidulated with acetic acid is treated with a solution of nitrate or acetate of uranium, a precipitate falls which is composed of uranium phosphate.

(*b*) When a soluble salt of uranium is added to a solution of potassium ferrocyanide, a reddish-brown precipitate or colour is developed.

Preparation of Standard Solutions of Uranium, &c.—Before

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* The reader is referred to the recent researches of Dr. Salkowsky, in Virchow's Archiv. Bd. 52, and of Maly, Pflüger's Archiv. 1872, vol. vi. p. 201.

preparing this solution, it is advisable to make a standard solution of a phosphate. For this purpose, 10·085 grammes of well crystallized sodium phosphate ($Na_2H\ P\ O_4 + 12\ H_2\ 0$) are dissolved in distilled water, and the solution diluted to one litre. Fifty cubic centimeters contain 0·1 gramme of $P_2\ 0_5$.

Then 100 grammes of sodium acetate are dissolved in 900 c. c., of distilled water, and 100 c. c., of acetic acid added.

The solution of uranium acetate is made by dissolving commercial uranic oxide in acetic acid, diluting and filtering; or, instead, a solution of uranium nitrate may be made by dissolving the crystallized salt in water, and diluting. The solutions are intended to contain 20·3 grammes of uranic oxide in one litre of solution.

Having obtained the solution of uranium acetate or nitrate, its strength is determined in the following manner: 50 c. c. of the standard solution of sodium phosphate are placed in a beaker, and 5 c. c., of the acid solution of sodium acetate added. The uranium solution is poured from an accurately graduated burette, until precipitation ceases. Then a few drops of a solution of potassium ferrocyanide are placed on a porcelain slab, and after each addition of uranium solution to the phosphate, a drop of the mixture is taken up by means of a glass rod and brought in contact with the ferrocyanide. As soon as an excess of uranium solution has been added, the characteristic reddish-brown colour of uranium ferrocyanide is observed.

It is convenient to graduate the solution of uranium so that 20 cubic centimeters shall be exactly equal to 50 c. c. of the standard solution of phosphate of soda, *i.e.*, to 0·1 gramme of $P_2\ 0_5$.

In analyzing urine by means of solutions of uranium, it is convenient to operate on 50 c. c. This quantity of urine is treated with the acetate of sodium solution and heated on the water-bath to a temperature approaching 100° C; it is then treated with the solution of uranium as previously described.

198. **Determination of the Quantity of Sulphuric Acid in Urine.**—The quantity of sulphuric acid in urine is best determined by precipitating with chloride of barium and weighing the dried and burned precipitate of barium sulphate; from this the amount of sulphuric acid can be calculated. It is usual to state the amount of sulphuric anhydride (SO_3) corresponding to the sulphuric acid existing in the urine.

For details as to the precautions to be used in determining the amount of sulphuric acid by precipitation, the student is referred to Fresenius's Quantitative Analysis. The manipulations involved in such an analysis, however simple it may be, can only be learned in a laboratory devoted to pure chemistry.

It has been suggested that the sulphuric acid in urine should be determined by means of a standard solution of chloride of barium; the method is one, however, which is tedious, and which cannot be recommended, even on the score of rapidity, as preferable to the one first described.

199. **Detection of Sugar in Urine.—It is still a matter of doubt whether the urine in health contains sugar; the processes which have been suggested, for the separation of this substance, by those who maintain its constant occurrence in healthy urine are, however, complicated; and as they have led to very various results in the hands of different observers, their consideration would be out of place in this book. (*See* Pflüger's Archiv. für Physiol. V. pp. 359 and 375.)

When present in abnormal quantities in urine, as in diabetes, glucose may be very readily detected. The following experiments will be sufficient to make the student acquainted with the more common reactions.

Experiment 1. Take 5 cubic centimeters of diabetic urine, or of a solution of grape sugar, and add to it two or three drops of a solution of copper sulphate, so that a very slight green tinge is perceptible; then add to the fluid a solution of caustic soda, or potash, until the precipitate of hydrate copper oxide, at first formed, is redissolved.

The fluid, which has assumed a blue tint, is now boiled, when an abundant precipitate of cuprous oxide falls; before this has separated, the fluid in which the precipitation is effected becomes opaque, and presents a reddish yellow colour. This is known as Trommer's test (*see* § 77 and § 12).

2. To five cubic centimeters of urine add nearly an equal volume of a solution of caustic soda, or potash, and boil. The fluid will assume at first a light yellow, then an amber, and lastly a dark brown coloration. This is known as Moore's test.

3. Some diabetic urine is mixed with a little brewers' yeast, and the mixture is poured into a test tube half full of mercury; the orifice of the tube is closed with the thumb, and the tube is inverted into a capsule containing mercury.

After a period of twenty-four hours, at ordinary temperatures, the test-tube will be found to contain large quantities of carbonic acid gas, which can be readily absorbed by passing up into the tube a fragment of caustic potash.

In addition to these tests, the student may with advantage determine, by means of a polariscope, that diabetic urine possesses the property of rotating the plane of polarized light to the right.

** 200. **Determination of the Quantity of Sugar in Urine.**—This may be best effected by one of the two following methods:—firstly, by determining to what extent a known depth of the saccharine fluid rotates the plane of polarized light to the right; or, secondly, by determining the quantity of urine which has to be boiled with a standard solution of a cupric salt, in order to reduce the whole of the copper to the condition of red cuprous oxide.

In order to determine the quantity of sugar by the last method, which is known as that of Fehling, we require to prepare a standard solution in the following manner:—34·65 grammes of pure and well crystallized copper sulphate are dissolved in about 160 cubic centimeters of water, and 173 grammes of Rochelle salts (tartrate of potash and soda) are dissolved in about 600 cubic centimeters of solution of caustic soda, having a specific gravity of 1120. The solution of sulphate of copper is added gradually to the alkaline solution of Rochelle salts, the fluid being continually stirred. A deep blue solution is thus obtained, which is diluted with distilled water to the volume of one litre. Ten cubic centimeters of this solution are reduced by 0·05 grammes of diabetic sugar.

The following is the process which has to be followed in determining the quantity of sugar in urine:—

The urine to be examined is diluted to a known extent; thus in the case of a diabetic urine, having a specific gravity of 1040, 100 cubic centimeters are diluted with distilled water to the volume of 1,000 cubic centimeters.

Ten cubic centimeters of the standard copper solution are then accurately measured out, and poured into a porcelain capsule. Forty cubic centimeters of distilled water are added, and the solution in the capsule boiled.

The previously diluted urine is then allowed to flow in from a burette; after a few cubic centimeters have been added, the fluid in the capsule is briskly boiled, and then the application of heat discontinued for a few seconds.

The solution, which, after the saccharine fluid has been boiled with it, assumes a red colour, deposits a red sediment of cuprous oxide, whilst the supernatant fluid retains a more or less blue colour, in consequence of a portion of the copper remaining in solution.

Successive portions of the diluted urine are then added, and the fluid boiled after each addition. As the operation proceeds, the addition of the diluted urine is performed with great care, only a few drops being poured in at a time. A point is at last reached when the bottom of the capsule is coated with a deposit of red cuprous oxide, and when, on tilting the capsule so as to bring the fluid which it contains over the clean white sides, no tint of blue is perceived.

The number of cubic centimeters of sugar solution added is then read off and marked. It is advisable, however, to pursue the operation one step further. A few more drops of diluted urine are added to the contents of the basin, which are again boiled, and if necessary, the addition is repeated until the boiled fluid becomes faintly opaque and of a yellowish colour. These appearances prove that a slight excess of sugar solution has been added. The number of cubic centimeters of diluted urine added is again read off. If the arithmetic mean of the first and second results be now taken, a number will be obtained which represents very accurately the volume of the dilute urine, in cubic centimeters, which was capable of reducing the whole of the copper in ten cubic centimeters of the standard solution employed. Now, as this volume of copper solution is reducible by exactly 0·05 grammes of diabetic sugar, this quantity must have been present in the volume of diluted urine made use of. An example will render the calculations required, intelligible :—The urine of a diabetic patient was found to have a specific gravity of 1035. 100 cubic centimeters were placed in a litre flask, and distilled water added until the fluid exactly measured 1000 c. c. Ten cubic centimeters of standard copper solution required 30·49 c. c. of the diluted urine in order to be completely reduced, or 30·49 c. c. of the diluted urine contained 0·05 gramme of sugar. As the urine had been diluted to ten times its original bulk, the same volume of the undiluted urine would contain ten times as much sugar, *i.e.*, 0·5 gramme of sugar. From these data we can easily ascertain, how much sugar was passed in the twenty-four hours.

Thus, if the quantity of urine passed in twenty-four hours, in the case under consideration, amounted to 3000 cubic centimeters, the amount of sugar passed during the same period would be at once found by the following proportion :—

$$30{\cdot}49 : 0{\cdot}5 :: 3000 : x$$
$$= 49{\cdot}19 \text{ grammes.}$$

The student, in carrying out the process just described, must be careful to dilute the urine to a sufficient extent. In cases where the percentage of sugar is very large, it is, for instance, convenient to dilute the urine twenty times instead of ten.

** 201. **Detection of Albumin in Urine.**—Except in very exceptional cases, which need not be alluded to here, the only albuminous body proper which appears in urine possesses the reaction of serum albumin. Accordingly, when albuminous urine is boiled, it is found to be coagulable, *i.e.*, the albumin separates in the insoluble form, and the coagulated albumin is insoluble in nitric acid. Nitric acid, when added alone to urine containing albumin, likewise precipitates that substance and the precipitate is not dispelled by heat. It must be stated, however, that in certain cases, when nitric acid produces a mere haze, this may disappear on boiling, although it be really due to a trace of albumin. Albuminous urine possesses the property of rotating the plane of polarization to the left.

* 202. **Determination of the Amount of Albumin in Urine.**—A known volume of the urine, say 50 or 100 cubic centimeters, is boiled; if the reaction is alkaline or neutral, a trace of acetic acid being previously added, the albumin separates freely and is collected on a weighed filter. The substance on the filter is repeatedly washed with boiling water, and after being allowed to drain, it is dried, first in a water oven at 100° C., and afterwards in an air oven at 120° C. The weight of the filter and albumin, minus the weight of the filter, furnishes us with the quantity of albumin (with adhering salts) present in the quantity of urine taken for analysis.

When a large number of determinations of albumin in urine have to be made, it is advisable to make use of the polariscope. The extent to which the plane of polarized light is rotated to the left bears a strict relation to the quantity of albumin present in a fluid, providing the depth of fluid examined be the same, and that no other substance (*e. g.*, sugar) be present, exerting an opposite action on polarized light.

** 203. **Detection of Bile-colouring Matter in Urine.**—When a large quantity of bilirubin is present in urine it may be separated from it by agitating the fluid with chloroform, decanting, evaporating the chloroform solution, dissolving the residue in pure chloroform, and allowing the fluid to evaporate spontaneously. In this way red rhombic prisms of bilirubin may be obtained.

In all cases where bile-colouring matter is present, we can detect it by the well known reaction with nitric acid (Gmelin's reaction). If strong nitric acid, containing nitrous acid, be added to a thin stratum of urine containing bile, in a flat porcelain dish, a succession of beautiful tints is perceived. The fluid is seen at first to be green, then blue and violet; it then assumes a rather dirty claret, and ultimately a dirty yellow colour (*see* § 135).

In cases where a very satisfactory search for traces of bilirubin is to be made, it is advisable to separate it from the urine, by means of chloroform, and then to test the evaporated residue with nitric acid. A property which is very characteristic of urine or other animal fluids coloured by bile pigment,is that of staining, of a yellow colour, linen which is moistened with it.

** 204. **Separation and Detection of Bile Acids in Urine.**—Four or five hundred cubic centimeters of urine are treated with acetate of lead until a precipitate ceases to fall, and then solution of ammonia is added. The precipitate is collected on a filter, washed with water, and allowed to drain. The filter paper, with the very bulky precipitate which it contains, is then boiled in a flask, with alcohol, and the solution is filtered whilst hot. A few drops of solution of sodium carbonate being added, the fluid is evaporated to dryness on the water-bath. The residue is boiled with absolute alcohol, and the solution is concentrated to a small volume. On adding an excess of ether to the alcohol, a precipitate occurs which consists of the soda salts of the bile acids, and which, if set aside for some time, often crystallizes.

This precipitate may be obtained by decanting from it the supernatant mixture of alcohol and ether. It is soluble in water; a few drops of the aqueous solution may be evaporated to dryness in a porcelain capsule and then subjected to Pettenkofer's test. This consists in adding a few drops of pure sulphuric acid, and then a trace of solution of cane sugar to it, and heating *very gently*. After some time, an exceedingly beautiful purple-violet coloration is developed.

Bile acids may be detected in the urine without previous separation by employing Strassburg's method (*see* § 140), but Hoppe-Seyler's method just described is much more reliable.

205. **Detection of Blood in Urine.**—Urine which contains blood, on being allowed to stand, usually furnishes a deposit in which characteristic blood corpuscles may be discovered without difficulty.

On examining such urine by means of the spectroscope, there is usually no difficulty in observing the spectrum of hæmoglobin or of hæmatin.

Urine which contains hæmoglobin furnishes when boiled, a precipitate of albumin and hæmatin.*

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* Although it has been considered advisable to devote some space to the mode of detecting a few of the more important abnormal constituents of urine, it would be beyond the object of this book to give a complete account of the properties of, and mode of separating, all the substances which occur in urine in a state of disease. Any additional information on these subjects is to be found in the very valuable "Handbuch der physiologisch- und pathologisch-Chemischen Analyse" of Professor Hoppe-Seyler, to which reference has been already made.

APPENDIX.

CHAPTER XXXIX.

PRACTICAL NOTES ON MANIPULATION.

206. **Manipulation of Glass Tubing.**—Most laboratories contain a glass-blower's table ; in its absence the mouth gas blowpipe must be used. The difficulty of keeping up a continuous blast of air with this instrument can be readily overcome by practice, provided that the orifice is not too wide. The blowpipe flame (fig. 325) consists of two parts, an inner blue cone (*a*) which is the deoxidizing or reducing flame, and an outer envelope (*b*) which surrounds it. The hottest part of the flame is a very little in front of the tip of the blue cone. The reducing flame is so called because the unburnt gases present in it have at that high temperature a great tendency to take oxygen from any substance containing it. In the outer envelope, on the contrary, the supply of oxygen is abundant ; it is therefore called the oxidizing flame. Ordinary English glass tubing contains oxide of lead : when it is heated in the reducing flame, black stains of metallic lead form on its surface. To avoid this, it should always be heated in the extremity of the outer flame. German glass is free from lead, and much less fusible than English glass, and is generally perferable to it. Tubes of German glass may be distinguished from English by looking through them lengthwise ; the former has a greenish colour, while the latter looks dark. In drawing out a glass tube so as to form a pipette (*see* fig. 326), care must be taken to soften the part to be drawn completely and equally, and to remove it from the flame before extending it. If this precaution is neglected, the drawn-out part will collapse and close. When heating a tube for the purpose of bending, it is important to use as low a temperature as is sufficient to soften it, and not to begin to bend until a considerable extent of the part to be bent is equally softened. For this reason, it is best to use a large flame (that from a gas jet being preferable to a Bunsen's lamp or blowpipe), in which the tube must be moved up and down until the object is attained. Before bending, it must of course be removed from the flame. In bending a *thin* tube, especially, if it be heated too strongly, it is difficult to avoid its becoming wrinkled at the bend. To avoid this, it is a good plan to close one end air-tight and blow in gently at the other during flexion. Large tubes are bent more easily by filling them with clean dry sand and heating them over incandescent charcoal, supported on wire netting. To seal a tube, it must be thoroughly softened at a short distance from its end, and drawn out quickly to a thread. The capillary part of a tube already drawn out is sealed instantaneously by directing the

point of a small blowpipe flame upon it and extending the heated part (fig. 327). To close a tube at its end, another piece of the same kind of glass must be joined to it by fusing the ends of both in the same flame. As soon as the joining has cooled slightly, the tube to be closed is heated again at a short distance from its end, and drawn out as before.

Annealing.—After glass has been strongly heated it must be allowed to cool as gradually as possible, in order to anneal it.

Manipulation of Corks.—To fit properly, a cork must be somewhat larger than the opening it is intended to fill. Before pushing it in, it should always be reduced by compression, either with a cork-squeezer or, in its absence, by rolling it on the floor (protected by a covering of paper) under the foot. For shaping corks, a shoemaker's knife which has been sharpened on a rough stone answers best. Any knife with a keen edge will do. To perforate a cork, a piece of brass tubing, the edge of one end of which is sharpened, is used. It is best to work the borer from the opposite ends, the two bore-holes meeting in the middle, as the holes always require finishing with a rat's tail file. A borer smaller than the intended channel should be used.

207. Solution and Ebullition.—The different solubility of various organic substances in reagents, such as water, ether, alcohol, acids, alkalies, and saline solutions, not only serves as a means of separating them from each other, but in many instances, as in the case of albuminous bodies, furnishes a characteristic by which one substance may be distinguished from others nearly allied to it. Tests are also more generally and conveniently applied to solutions than to bodies themselves. Solution takes place more readily when the body to be dissolved is finely divided. Dry and hard substances are therefore generally pulverized by pounding and rubbing in a Wedgwood mortar. If too large to be conveniently triturated at once, they may be previously broken in an iron mortar, or by wrapping them loosely in brown paper and pounding them with a hammer. If the substance is constantly shaken or stirred about so as to bring it continually into contact with fresh portions of the solvent, it will dissolve much more quickly than if allowed to remain at rest.

For preparing Solutions.—A beaker is for most purposes the most convenient vessel, as its contents can be stirred at the same time that it is subjected to heat, which always accelerates solution. To avoid risk of fracture, the beaker must not be heated over a naked flame, but must be placed on a piece of wire gauze or sand bath (fig. 328), supported on a tripod. Flasks may be employed instead of beakers for solution or boiling when stirring is not required. They have the advantage of preventing loss of fluid during the process of ebullition, as any particles which spurt up are caught against the sides of the flask, especially if it is placed in an inclined position, instead of falling outside as in a beaker.

To prevent Loss by Evaporation.—Various methods may be used. One of these consists in placing a small funnel in the mouth of the flask; the fluid condenses in the funnel and runs back into the flask. Another method is to close the neck of the flask with a cork, through which a wide glass tube, drawn out to a capiliary opening at its upper end, is passed. A considerable part of the vapour passing from the boiling liquid is condensed in the tube and falls back into the flask. If the boiling is long continued, the tube gets very hot and a great deal of vapour escapes. To avoid this, the escape tube is prolonged and surrounded by a Liebig's condenser, for which purpose it must be bent at an angle of about 120°, as seen in fig. 329.

To exhaust a substance with ether, the ether and the substance should be placed in one flask, with which a second is connected by a bent glass tube which passes through the corks of both. The tube which scarcely projects beyond the under surface of the cork in the first flask, reaches to the bottom of the second. The first flask being then placed in a beaker of warm water and the second in cold, the ether distils over from the former into the latter and is condensed. When a large quantity of the ether has passed over, the flasks are transposed, when the whole of the ether rushes back into the first flask. The process may be repeated indefinitely.

In connection with this subject, an arrangement may be described which is chiefly used for washing precipitates. It is also applicable for the purpose of replacing loss by evaporation when liquids are boiled, or to keep the water at a constant level in a water-bath. (*See* fig. 331.) It consists of a large flask, *a*, fitted with a cork, through which pass two tubes. One of these, *b*, *c*, is straight and open at both ends; the other, *d*, *e*, *g*, *f*, is bent so as to form a syphon, the limbs of which are of equal length. Both ends of *d*, *e*, *g*, *f*, are at a somewhat lower level than the lower end of *b*, *c*. The end is placed in the funnel or water-bath at such a height that the level of the lower end of *b*, *c*, coincides with that at which it is desired that the surface of the fluid shall remain. The effective difference in the limbs of the syphon is the space between *c* and *d*. Whenever the surface of the liquid in the funnel or bath is on a level with *c*, the tube *d*, *e*, *g*, *f*, ceases to act as a syphon; but as soon as it falls, *d*, *f*, again acts, and liquid runs into the funnel till the surface is again level with *c*.

208. **Evaporation.**—Evaporation of watery liquids is usually conducted in shallow basins of Berlin porcelain, heated either directly in a sand-bath or over a water-bath. An ordinary saucepan answers perfectly as a water-bath. (*See* fig. 330.) If the naked flame is used, it ought not to be allowed actually to touch the bottom of the basin. The process is greatly accelerated by constant stirring.

If it is important that none of the substance be lost, the liquid must not be heated to boiling, as it is then apt to spirt over the sides. In evaporating a solution to dryness, its surface often becomes covered towards the end of the process with a pellicle, which hinders the vapour below from escaping easily, and thus both retards evaporation and causes the vapour to issue in jets which may occasion loss of material. The formation of the pellicle is best prevented by stirring the fluid constantly with a glass rod. It may also be prevented by covering the evaporating basin loosely with another somewhat smaller one, or with a concave glass with the concavity downwards, but this retards evaporation. Solutions in alcohol, ether, and chloroform, must be evaporated in beakers. Solutions in the two latter menstrua must never be evaporated over a naked flame, but always on a water-bath, as their vapour is inflammable.

Evaporation at a Constant Medium Temperature.—It is sometimes desirable to evaporate a liquid at a constant medium temperature, such as 40° C. This may be done roughly by placing the evaporating basin in a sand-bath, and carefully regulating the size of the flame by a thermometer. Unless, however, it is constantly watched, the temperature is apt to rise or fall too much, and the solution may get spoiled. This difficulty is avoided by using a water-bath heated by a gas-lamp, which is connected with a Bunsen's gas-regulator. For this purpose I find a water-bath of the accompanying form (fig. 331) a convenient one. It is made of galvanized zinc, is eleven inches in diameter, and five deep. At one side it bulges out, and in the projecting part thus formed the thermometer and regulator are placed. The top of the bath is covered by a zinc plate perforated by several large holes, in which evaporating basins may be put; or by a series of concentric copper rings, one or more of which may be removed so as to accommodate evaporating basins of different sizes. The regulator, as modified by Geissler (fig. 332), consists of a wide glass tube, *a*, divided into two parts, an upper and a lower, by a horizontal septum. In the middle of the septum is an opening, from which a tube runs down nearly to the bottom of the lower division.

The tube is closed by a perforated cork or india-rubber stopper. Through this passes a tube, B, with a horizontal limb, E. Inside B is a smaller and shorter tube, C, which has a very small opening opposite D. The sides of B and C are luted together with cement at F. In using this regulator, a quantity of mercury is poured into A, and of course runs down into the lower division, partly filling it, and partly compressing the air it contains.

The mouth of A is then closed by the cork, and the tube C connected by india-rubber tubing with a gas-pipe, and the tube E with a small gas-burner. The gas passes down the tube C through its lower open end, up again between it and B, and out at E, and thence to the burner. The regulator and a thermometer are then immersed in the water-bath, the gas lighted, and the bath warmed till the thermometer indicates 40° C., or any other desired temperature. The tubes B and C are then pushed down till the mercury touches the lower end of C and closes it. The gas is thus prevented from passing onwards to the burner, and the flame would go out entirely were it not that the small hole in C, opposite D, allows sufficient gas to pass through it to preserve the flame from being completely extinguished. As soon as the flame is thus diminished, the water-bath and the regulator immersed in it begin to cool, and the mercury, and still more the air in the regulator, consequently contracts. The mercury, therefore, sinks, and leaves the mouth of C open, so that the gas again passes freely through it, the flame increases, and the temperature of the bath again rises. The mercury and air again expand; and as soon as the temperature is reached to which the regulator was adjusted, the mercury again closes the mouth of C, and cuts off the gas till the temperature again falls. In this way the temperature may be kept for months at 40° without varying much more than half a degree. Unless the mercury is very clean, however, it will adhere slightly to the lower end of C, and the variations will thus be greater. The water in the bath must also be kept at a constant level, as otherwise the part of the regulator heated by it is sometimes greater and sometimes less. The mercury consequently does not always expand in the same proportion to the rise in temperature of the water in which it is partially immersed, and variations of several degrees may thus be produced.

209. Precipitation.—In precipitating a substance by the addition of another, the reagent is generally added a little at a time, and mixed by means of a stirring rod, till a further addition of the reagent produces no perceptible increase in the amount of the precipitate. In order to ascertain that the precipitation is complete, a little of the liquid is tested by throwing it on a filter, and the reagent added to the clear filtrate. If no further precipitate occurs, the precipitation is complete; but if one is formed, the filtrate is again mixed with the rest of the fluid and the process repeated.

210. Washing of Precipitates on Filters. — Precipitates are generally collected on a filter and washed by directing a stream of water or alcohol on them by means of a wash-bottle. The filter should never be filled up to the top, as the upper part of the precipitate cannot then be properly washed. It is always advisable to let the precipitate settle in the beaker, and to allow the clear liquid to pass through the filter before throwing the precipitate itself upon it; and the whole of the fluid from which the precipitate has subsided must be allowed to pass through the filter before the washing is begun. A stream of water is then directed on the part of the precipitate nearest the edge of the filter, by which it is gradually washed towards the centre. The stream should not be too strong, nor should it strike the filter or precipitate perpendicularly, as it is then apt to scatter the precipitate or tear the filter. When the filter is nearly full of water, the whole should be allowed to run through, and the washing again repeated.

Washing of Precipitates by Decantation.—When a precipitate subsides quickly, it is more readily washed by decantation than on a filter. Granular and gelatinous precipitates are not easily washed completely on a filter, and it is better to wash them as well as possible by decantation, and to finish the operation on a filter. In washing by decantation, the precipitate is placed in a tall beaker, and stirred well with a quantity of water, alcohol, or other washing liquid. It is then allowed to subside, and the supernatant liquid carefully poured off or removed by a syphon (*see* fig. 333); this is repeated till the washing is complete. In order to prevent any of the precipitate being carried off in the washing and lost, the liquid used for washing may be collected and passed through a filter. Any part of the precipitate retained by the filter may then be washed, and the rest of the precipitate added to it.

211. **Filtration.**—Filtration is the separation of insoluble substances from liquids by passing the latter through a porous material which keeps the former back. When the substance to be removed is in large pieces, or when the liquid is thick and viscid, and will not pass easily through paper, it may be filtered through linen or gauze. The linen may either be stretched over the mouth of a beaker or placed in a porcelain strainer in the form of a hollow cone, with numerous perforations near its apex. The removal of the last portions of the liquid may generally be hastened by squeezing the linen either with the hand or in a press (fig. 334). Fine precipitates are usually separated by filters of unglazed porous paper, made specially for the purpose. To make a filter, take a round or square piece of paper of the proper size, and fold it twice at right angles. If a square piece has been used, it must now be cut round. Open it in the form of a cone, and place it in a funnel. If the funnel is of proper form (its sides sloping at an angle of 30° to its axis), the filter will fit it exactly. If it does not, the angle at the apex of the paper cone must be modified. The filter should always be a little smaller than the funnel, and never project above its edges. Before pouring in the liquid to be filtered, the paper must be moistened with distilled water, alcohol, or ether, according as the liquid is aqueous, alcoholic, or ethereal. If this is not done, the first portions of the fluid which pass through are apt to be muddy, but they may be cleared by pouring them back on the filter and making them pass through a second time. To avoid breaking the filter at the apex, the liquid should be poured on it so as to fall on its sides, which are supported by the funnel, and the stream directed by a glass rod The filtrate is generally collected in a beaker; it is well to let the end of the funnel touch the side of the glass, so that the liquid may run down it without splashing. If the filtrate only is wanted, filtration may be quickened by using a ribbed or plaited filter. To make this, take a circular piece of filter paper and fold it into quadrants, and then into half quadrants, making all the folds towards one side. Then make a fold towards the other side between each two of those already made, and push the paper into the funnel, pressing the point down into the neck of the funnel; then pour in the liquid, when it will open completely. Instead of this, three glass rods, bent at the top so as to hook on to the edge of the funnel, may be laid inside it at equal distances from each other. These are useful both in quickening the filtration and in supporting the bottom of the filter, especially when the funnel is badly made and its tube is too wide at its junction with the cone. When albuminous liquids are filtered through paper, the pores become very quickly choked up, and it is therefore better to use a number of small filters than one large one; and when the fluid ceases to pass through one set of filters, to pour it into fresh ones.

Filtration by Bunsen's Pump.—Filtration may be much accelerated by filtering the liquid into a partial vacuum. This is done by fixing the funnel air-tight in one neck of a Woulfe's bottle, and exhausting the air through the other by an ordinary exhausting syringe. It can, however, be more conveniently effected by means of a Bunsen's water air-pump (fig. 335).

The principle of this instrument is the same as that of Sprengel's pump, with this difference, that water is substituted for mercury. It consists of a wide air-tight tube, through which water descends in a constant stream to a depth which (if it is desired to produce a complete vacuum) must not be less than thirty-two feet. Into the axis of this tube, close to its upper end, a second tube of much smaller bore projects, the open end of which looks downwards, *i.e.*, in the direction of the stream. Through this tube, if it is open, air is constantly drawn; any closed cavity with which it is in air-tight communication is rapidly exhausted. It may thus be used either as an aspirator or as an air-pump. If, however, the height of the column of water is less than thirty-two feet, its exhausting power is limited to the production of a diminished pressure, which is expressed by the difference between the height of the column and thirty-two feet. The usual way of employing it in filtration is to attach the extraction tube H to a piece of bent glass tubing, which passes through an india-rubber stopper in one neck of a Woulfe's bottle, in the other neck of which a funnel is fixed in a similar manner. The air inside the bottle being exhausted by the air-pump, the fluid is forced rapidly through the filter by the pressure of the external atmosphere. I find it more convenient to use a strong bell jar, with a tubular opening at the top. Into this opening an india-rubber stopper, which is perforated for the funnel and exhausting tube, is fitted. The beaker in which the filtrate is to be received is placed on a ground-glass plate. The ground edge of the bell jar having been smeared with resin ointment, it is set on the plate in such a position that the funnel is exactly over the beaker. The fluid is then poured into the filter, and the air exhausted from the bell jar. The pressure of the air would force the liquid through the filter and tear it away unless it were supported in some way. This is done by taking a semi-circular piece of platinum foil of suitable size. A snip having been made at the centre of the straight edge, and at right angles to it, the bit of foil is heated in the blow-pipe flame, and allowed to cool. It can then be smoothed out, bent at the snip, and the edges brought together so as to overlap each other slightly. The hollow cone thus formed is next placed in an iron mould with a conical cavity, and pressed firmly in with a conical plug. The funnel used must be chosen with sides sloping at the proper angle, and the tube must not be too wide at the junction with the cone. The platinum foil is placed in the bottom of the funnel, and pressed with the finger, so as to fit the funnel smoothly. Instead of platinum foil, fine wire gauze or parchment paper is sometimes used. The filter is then folded and placed with its apex resting in the platinum, moistened with water, and pressed gently against the sides of the funnel so as to make it fit tightly to it, and prevent air from getting down into the receiver between them. Milk, albuminous solutions, and glycerin can be filtered much more readily through porous earthenware than through paper. For this purpose the top of a porous cell, such as is used for galvanic batteries, is closed by an india-rubber cap with two openings. One of these is connected by a short glass tube and strong india-rubber tubing with the pump. Through the other a glass tube passes nearly to the bottom of the cylinder, and is closed at its upper end by a piece of india-rubber tubing and a strong clip. This serves as a pipette to remove a little of the fluid occasionally from the cell for the purpose of testing it. The cell is placed in a glass cylinder, little more than wide enough to admit it, and the fluid to be filtered is poured into the cylinder until it covers the lower part of the india-rubber cap. The air being then exhausted from the cell, the fluid filters into it from the cylinder. Instead of cells, cones of porous earthenware may be used as filters. A short piece of wide india-rubber tubing is stretched over the top of a funnel, and into its upper end, which lies flat across the opening of the funnel, a porous cone is inserted (*see* fig. 335). In order to keep liquids hot during the process of filtration, Plantamour's funnel is used. This is a hollow funnel of copper containing water, which is kept hot by a flame applied to a projecting part. A better plan is to use a water-bath with a funnel-shaped opening in it (fig. 336). This has the advantage that it may be kept at any required temperature with the aid of a Bunsen's regulator.

212. **Dialysis.**—Almost all crystalline bodies, with the notable exception of hæmoglobin, pass readily, when in a state of solution, through animal membranes or through vegetable parchment. The great majority of non-crystalline bodies, such as albumin, do not pass through at all, or only with very great difficulty. In this way the diffusible may be separated from non-diffusible substances. Such a separation is termed dialysis. Graham, the discoverer of the process, gave to the diffusible bodies the name crystalloids, to the non-diffusible the name colloids, as he thought all crystalline bodies diffused and all non-crystallizable did not; but these names are open to objection since the discovery that hæmoglobin will not diffuse, although it forms crystals, while peptones diffuse, although they do not crystallize. Dialysis is effected by placing the liquid which is to be dialysed in a cylinder, of which the bottom consists of vegetable parchment. This cylinder, called a dialyser, is then placed in a shallow vessel containing distilled water. The diffusible substances pass through the parchment into the water, while the non-diffusible remain behind. Two forms of dialyser are in ordinary use. For dialysing small quantities, bell-shaped glass jars are used. For quantities of seven or eight ounces or upwards, a dialyser is employed which consists of two gutta-percha hoops, one of which is two inches deep, the other only one. The deeper hoop is slightly conical, so that the other hoop slips over its smaller end.

Before using this contrivance, both hoops must be washed thoroughly with distilled water. A piece of vegetable parchment, about three inches wider than the smaller end of the deep hoop, must then be steeped for a minute in distilled water and stretched over it. After applying the edges of the parchment carefully to the outside of the smaller hoop, the larger one is slipped over it, so as to fix it tightly. The dialyser must next be tested, to ascertain that the parchment is free from holes. It must be filled to the depth of a quarter of an inch with distilled water, and placed for a short while on a piece of blotting-paper. If there are any holes in the parchment, the water will come through and leave a wet spot on the blotting-paper, in which case either a fresh piece should be put on or the holes closed up. This may be done by sticking a piece of vegetable parchment over the holes on the under surface of the dialyser with white of egg, and then passing a smooth hot iron over the patches. This done, the dialyser must be again tested. After having been ascertained to be perfect, it may be filled; the liqui to be dialysed must not cover the bottom to a greater depth than half an inch. It must then be floated in about five times as much water as it contains of liquid (fig. 337), and gently agitated from time to time.

The bell-shaped dialysers are used in the same way, but the paper is fixed over the wide end with a piece of fine cord, and the dialyser, instead of being floated on the water, is suspended so that the parchment is *just* below the surface. This is effected by strings which pass from its neck to a glass rod laid over the mouth of a cylindrical glass jar containing the water (fig. 338). Diffusion is promoted by using a large surface of parchment, or by frequently gently shaking the dialyser. The process may be further accelerated by heat and by evaporation, for which purpose the basin containing the dialyser may be advantageously placed in the warm chamber or bath at a temperature of 37° C.

213. **Drying.**—Glass vessels, in which substances are to be weighed, are

dried by heat. In the case of flasks and tubes, this may be done by warming them over the flame of a spirit lamp, then blowing air through them with the bellows. For most purposes the *hot-air bath* is used—a copper vessel either cubical or cylindrical in shape, and provided with a door or movable cover (fig. 339). It is heated by a lamp or burner, and must be furnished with a thermometer, so fixed as to indicate the temperature of the air of the chamber. For all purposes which require a temperature not exceeding 100° C., the hot-air bath must consist of two casings, the space between which is filled with water.

Drying and Cooling over Sulphuric Acid.—When substances, especially hygroscopic powders, are dried in the air-bath and then allowed to cool, they take up moisture and gain weight. To prevent this, they must be allowed to cool under a bell jar, under which is a dish containing sulphuric acid (fig. 340). The acid absorbs moisture with avidity, and keeps the air under the jar dry. The acid may be placed in a shallow dish, and the substances to be dried supported over it on a sheet of perforated zinc, which rests on the edges of the dish or on a small tripod. Another method is to put the acid in a beaker, covered with a ground-glass plate greased at the edges, and to support the crucible on a leaden support; the support is made of a bit of strong leaden wire by bending one end of it into a circle which lies at the bottom of the beaker, and the other end into a smaller circle which rises above the surface of the acid and holds the crucible. To prevent dried hydroscopic substances from taking up moisture during weighing, they should not be placed in an open vessel, but enclosed between two watch-glasses held together by a spring.

When it is desired to dry substances without the aid of heat, they are to be placed under the receiver of an air-pump and over sulphuric acid, as just mentioned.

Precipitates may be rapidly dried by supporting the funnel containing them over a very small flame by means of a beaker with the bottom out, a triangle of iron wire and a piece of wire gauze, arranged as in fig. 341.

214. **Ignition.**—Substances are exposed to a red heat in order to dry them thoroughly, to drive away volatile matters, or to burn off organic constituents, and allow the fixed inorganic solids to be determined. A small quantity of a substance may be ignited on a piece of platinum foil or in a platinum spoon, larger quantities in porcelain or platinum crucibles. Platinum vessels should not be used if the substance to be ignited contains iodine, bromine, phosphorus, or easily reducible metals, such as copper, lead, silver, gold, or tin. When precipitates collected in a filter are ignited, they must be first carefully dried. The crucible is then to be placed on a piece of glazed paper, the precipitate loosened from the filter by rubbing the sides together, and then shaken gently into the crucible. The filter is then either folded and placed in the crucible, or it is set fire to and held over it by a pair of forceps, so that the ashes may fall into it. Any ashes or precipitate that has fallen on the paper having been collected and added to the rest, the crucible is placed in a triangle of platinum wires stretched on a larger one of iron wire (fig. 342), and heated over a Bunsen's lamp. The cover should be laid on the crucible at first to prevent any loss, and the heat raised very gradually. The cover may be removed during part of the process to allow freer access of air, but towards the end it should again be replaced so that the heat within the crucible may become greater. With the same view, the blow-pipe flame may be substituted for that of the Bunsen's burner. The crucible is then allowed to cool somewhat on the triangle, but while still warm must be placed over sulphuric acid, and left there till cold. The weight of ash left by a good filter is very inconsiderable; but it may be ascertained by burning a dozen filters and dividing the weight of the ash by the number. Filters may be almost completely deprived of ash by extracting

them with dilute hydrochloric acid, and washing them with water till the acid reaction completely disappears.

215. **Weighing.**—The balances most useful in a physiological laboratory are a fine analytical balance to carry 100 grammes in each pan, and turn easily with half a milligramme or less, and a large balance to carry seventy kilogrammes, and turn with a few grammes. Fine balances are always protected by glass covers, to prevent the access of dust and protect the instrument from draughts of air, &c. Inside this, a vessel containing chloride of calcium is often placed to keep the air dry. The doors of the case should be only opened when the substance or weights are to be adjusted, and should be closed while the beam is oscillating. It is convenient to lay the weights on a sheet of paper on the floor of the balance, and to mark the weight of each on that part of the paper where it lies. They must never be touched with the fingers, only with forceps. It is advisable always to place the weights in the same pan (the right) of the balance, and the substance to be weighed in the other. The placing of heavy weights on a fine balance should be avoided, even though they may not exceed the weight which the instrument is constructed to carry. Nothing should be placed on the pans or taken from them while the beam is oscillating. It is not necessary to wait each time till the index stops moving in order to see whether there is any difference between the weights in the pans; for this is ascertained much more exactly by observing whether the index oscillates farther on one side of the zero mark than on the other, than by noticing its position when at rest. After weighing, add together the weights which are absent from their places on the paper. Note down the weight *at once,* and check it by adding the weights together as they are lifted from the pan and replaced. No weight should ever be allowed to remain on the balance after weighing. Substances are generally weighed in watch-glasses, small crucibles or small flasks. These may be either weighed separately, and their weight deducted from the total weight, or they may be counterpoised. To save the trouble of weighing them each time, they may be carefully weighed once for all, and their weight noted and marked on them with a diamond, or, if they are of porcelain, in ink. When a crucible with its lid is used, it is usual to put corresponding marks on the crucible and its lid, so that the same may be used each time. Counterpoises may be made in various ways. The most convenient is to choose a piece of brass of about the size of the brass weight which corresponds most closely to the weight of the vessel to be counterpoised, and reduce it by careful filing till the weights are exactly equal. If only required for temporary use, a pill-box partly filled with small shot will suffice.

216. **Specific Gravity.**—The specific gravity of a solid or liquid is its weight compared with that of an equal bulk of distilled water. Water and other liquids, however, shrink when cooled, and expand when heated, so that the weight of a given bulk varies with the temperature. If a vessel containing, for example, a cubic inch is filled with a fluid at a moderate temperature and cooled, the liquid will shrink, and more must be poured in to fill up the space. If, on the contrary, it be warmed, the liquid will run over. The weight of the cubic inch of cold liquid will be greater than that of the liquid at the original temperature by the quantity poured in, while that of the hot liquid will be less by that which has run over. It is therefore absolutely necessary to compare the weights of bodies at the same temperature. Specific gravities are in this country estimated at 15° C. or 60° F.

Specific Gravity of Liquids.—The specific gravity of a liquid may be ascertained by the use of the specific gravity bottle, the hydrometer, or specific gravity beads.

The Specific Gravity Bottle.—This is a small bottle which contains a known volume of liquid; one form of bottle (fig. 343) contains its proper quantity when it is filled perfectly full, another form (fig. 344) when filled up to a mark on the neck, which is long and thin. The bottle having been charged with the liquid, of which the specific gravity is to be determined, the weight of its contents is determined by the balance, for which purpose it must first be counterpoised. The quotient obtained by dividing the weight of the liquid by the weight of the same bulk of water at the same temperature is its specific gravity. It is difficult to fill an ordinary bottle completely and to put in the stopper without getting in an air-bubble, which would of course alter the weight of the contents and so give false results. To obviate this difficulty, the stopper of a specific gravity bottle has a hole bored up through its middle, so that when the bottle is filled and the stopper put in, any air or fluid that may be present in the neck passes up through the hole, and thus both the bottle and the hole in the stopper are completely filled with fluid. Before weighing the empty bottle or making a counterpoise for it, it must be thoroughly dried. Specific gravity bottles of this kind are usually constructed to contain from 50 to 100 grammes of distilled water at 15° C. Counterpoises are always sold with them. Before using them, the accuracy both of the counterpoise and of the capacity of the bottle must be tested. For the latter purpose, the bottle must be filled and then immersed in a beaker containing distilled water at a temperature a few degrees higher than 15° C., and allowed to remain until a thermometer standing in the water indicates that the required temperature has been reached. The bottle must then be removed from the beaker and weighed against the counterpoise, its outside having been first carefully wiped dry. The weight is that of the distilled water contained in the bottle at 15° C. In weighing the contents of the bottle when charged with any liquid of which the specific gravity is to be determined, the same method is to be followed, with the exception that the bottle must not be completely immersed in the liquid contained in the beaker. If then w indicate the weight of the water and w' that of the same volume of the other liquid at the same temperature, its specific gravity $= \frac{w'}{w}$.

Sometimes it is difficult to get a sufficient quantity of liquid to fill the specific gravity bottle just described. When this is the case, a specific gravity bottle may be made out of a test-tube, by drawing it out, as in the accompanying figure (fig. 345), and then flattening the bottom so as to make it stand by heating it and pressing it against a piece of iron. A scratch is to be made on the narrow part of the neck, up to which the bottle is to be filled with water at 15° C., and weighed against a counterpoise as before. In all other respects the procedure is that which has been already described.

The Hydrometer.—The hydrometer is an elongated glass bulb which is weighted at one end so as to make it float upright, and is prolonged at the other end into a stem, graduated in such a manner that the number of the division up to which the instrument sinks expresses the specific gravity of the liquid in which it is placed. As every instrument reads accurately only at the temperature for which it is constructed, the liquid must be brought to the proper temperature before the instrument is used. In using the hydrometer, the liquid must be placed in a cylindrical glass vessel, deep enough and wide enough to allow the instrument to float freely in it without coming in contact with the sides or bottom. The froth, if any, is then to be removed from the surface with a piece of blotting-paper, and the hydrometer allowed gently to sink into the liquid. The mark on the scale, which coincides with its surface, indicates the specific gravity. To read this correctly, the eye must be brought to a level with the surface of the liquid. When this is the case, the surface presents the form of a meniscus, assuming the aspect of an ellipse when the eye is either raised or lowered. To ensure accuracy, the reading should be repeated once or twice, the hydrometer being pressed down in the liquid between each two observations.

Specific Gravity of Solids.—The specific gravity of a solid mass, the substance of which is insoluble, is ascertained by weighing it first in air and then in water. The difference between these weights is equal to the weight of its own bulk of the water which it displaces. The specific gravity is therefore got by dividing the weight of the solid in air by the difference between its weight in air and water. The weight of solids may also be ascertained by immersing them in fluids of known density till they float. Thus the best way of ascertaining the specific gravity of the substance of the brain, or any other organ, is to prepare a graduated series of solutions of common salt of different densities, and to immerse the solid, first in one, and then in another, till a solution is found in which it floats indifferently at any height.

217. Volumetrical Analysis.—For volumetrical analyses, measuring flasks, measuring glasses, pipettes, burettes, and other accessory apparatus are required.

Measuring Flasks.—These flasks, of the form shown in fig. 346, are used for dissolving substances for the preparation of standard solutions, &c. They should have tolerably wide mouths, and be furnished with well-fitting stoppers, so that they may be shaken without risk of loss. The graduation mark should be just below the middle of the neck. Flasks are used of capacities varying from 100 centimetres to a litre. *Graduated cylinders*, such as that shown in fig. 347, generally called test-mixers, are used for the same purpose.

Pipettes.—A pipette is a glass tube of the shape shown in fig. 348, and when filled up to the mark on the neck it should deliver the exact quantity of fluid which is marked upon it. Some pipettes are graduated so as to let the exact quantity run out by its own weight; others, to deliver the right amount only when the liquid is blown forcibly out. The former are to be preferred. Another kind of pipette is graduated along the greater part of its length, so as to deliver different quantities at will, but it is not so accurate as the others. In using pipettes, the liquid to be measured is to be put into a test-glass or small beaker; the lower end of the pipette is then immersed in the liquid, which is to be sucked up till it stands somewhat above the mark on the neck of the pipette. The upper end of the pipette must then be quickly covered with the moistened tip of the forefinger, so as to prevent the liquid from flowing out. The mark on the neck is next brought to a level with the eye, and the tip of the finger gently raised so as to allow the liquid to escape slowly till it stands opposite the mark. It is then allowed to run out into a clean beaker, and the last few drops removed from the point of the pipette by touching it against the side of the beaker.

Burettes.—These are used for delivering standard solutions. There are several forms of burette, but the most convenient is that of Mohr. It consists of a graduated tube, to whose lower end an india-rubber tube is attached, which can be opened and shut by a spring clip (fig. 349), so that the operator can let the solution run out or stop it at will. The burette is supported in an upright position on a stand made for the purpose (fig. 352). To prevent dust getting in, a polished marble should be placed on its upper end. In many cases the spring clip answers well, but when nitrate of mercury is used it attacks the clip, and bichromate of potash destroys the india-rubber. For such liquids a burette furnished with a glass stopcock is to be preferred. A burette should be filled by allowing the liquid to flow gently into it while it is held in an inclined position in the hand till it stands above the zero mark. The instrument is then replaced. If any air-bubbles are present, they must be allowed to break, or removed by a glass rod. The solution is then allowed to flow out till its level corresponds to the zero mark on the burette.

Rules for Reading Burettes and other Graduated Instruments used in Volumetrical Analysis.—When liquid is contained in a narrow tube, its surface is higher at the edges where it touches the glass than elsewhere; and if we examine the curved surface by transmitted light, it seems to be formed of several zones or bands, the lowest of which is dark (fig. 350). To avoid errors and uncertainty, the under border of the dark zone is always regarded as indicating the level at which the liquid stands. In reading, the eye must of course be exactly level with the surface, otherwise the reading will be either too high or too low. The under surface of the liquid is more easily seen if a card, with its under half blackened, while its upper half remains white, be held behind the liquid, so that the division between the black and white parts is about one-eighth of an inch below its surface. The lower surface of the liquid then seems to be bounded by a sharp black line (Sutton). Burettes may be read very easily and with great accuracy by using Erdmann's float (fig. 351). This is an elongated glass bulb, weighted with mercury at its lower end, so that it floats upright. Its diameter being a very little less than the calibre of the burette which contains it, it moves freely, but at the same time steadily, up and down. A horizontal mark round its middle is taken as indicating the height at which the liquid stands, the absolute height being disregarded.

Litmus Solution.—The solution used in the neutralization of albuminous liquids is prepared by dissolving a little litmus in distilled water, decanting the liquid from the sediment and diluting it as required. For determinations of the strength of acid, the litmus solution is made by putting 10 grammes of solid litmus into half a litre of distilled water, letting it stand for a few hours in a warm place, decanting the clear fluid, adding a few drops of dilute nitric acid so as to produce a violet colour, and preserving it in an open bottle with a narrow neck. If the colour should at any time partially disappear, it may be restored by exposing the liquid to the air in an open bottle (Sutton).

Volumetric Solution of Soda.—Fill a burette with solution of soda, and cautiously drop this into 6·3 grammes of purified oxalic acid in crystals, quite dry but not effloresced, dissolved in about 70 c. c. of distilled water, until the acid is exactly neutralized, as indicated by litmus. Note the number of grain measures (n) of soda solution used, and having then introduced 900 cub. cent. of it into a graduated jar, augment this quantity by the addition of water until it becomes $\frac{900 \times 140}{n}$ cub. cent. If, for example, $n = 93$, the 900 cub. cent. should be augmented to $\frac{900 \times 100}{93} = 967{\cdot}7$ cub. cent. 100 cub. cent. contain $\frac{1}{10}$th of an equivalent in grammes (4 grammes) of hydrate of soda, and will neutralize $\frac{1}{10}$th of an equivalent in grammes of an acid.

Soda solution for estimating the acidity of gastric juice is made by diluting 100 c. c. of the above solution to the bulk of a litre.

218. Polariscope.—There are several organic substances whose solutions possess the power of circumpolarization, *i.e.*, of rotating to one side or another the plane of polarization of a ray of polarized light passing through them. Some of them, such as glucose, cane-sugar, and tartaric acid, turn it to the right hand, while others, such as albumin, uncrystallizable sugar, and oil of turpentine, turn it to the left. As the amount of rotation increases in proportion to the concentration of the solution and the thickness of the stratum through which the ray passes, it is easy to ascertain the quantity of a substance held in solution by simply observing the extent to which a ray is rotated in passing through a stratum of a definite thickness. The apparatus used for this purpose is shown in fig. 353. It consists of a stand in which are placed two Nicol's prisms, *a* and *b*.

The prism *b* is fixed, but that at *a* is movable, and the extent to which it is rotated is indicated on a graduated circular disk *s s* by an index *z*. When the two prisms are placed exactly in the same position, the ray which has been polarized by *b* passes readily through *a*, and the field of vision of an observer looking into the instrument at *a* is illuminated. As *a* is turned round on its axis, the field becomes dimmer and dimmer till the two prisms are turned crosswise to each other, when the polarized ray by *b* is entirely stopped by *a*, and the field consequently becomes quite dark. At this time the index stands at zero. If a glass tube containing a solution of sugar or albumin is then placed in the space *o o*, the polarized ray will pass through it, and in doing so will have its plane of polarization more or less rotated, so that it will no longer be entirely stopped by the prism *a*. In order, therefore, to stop it again and produce a dark field, this prism must be rotated to a corresponding degree, and the extent of rotation is read off on the graduated disk. As it is difficult to determine exactly the position of *a*, at which the field is darkest, some additions have been made to this instrument by Soleil and Ventzke, which make their saccharimeter more complicated, but greatly increase its exactitude. The first of these is a plate of quartz, *q*, composed of two pieces whose line of junction is exactly in the middle of the field of vision. One piece rotates light to the right hand, while the other turns it to the left. When a solution of sugar is placed in the space *o o*, it increases the action of that half of the plate which rotates to the right, and lessens the action of the other half which rotates to the left, and the two halves of the field of vision become of a different colour. This difference can be removed by turning the prism *a*, but this is more easily effected by means of the compensator *n*. The chief parts of this are figured separately. It consists of two equal prisms (*r* and *r′*) of left-handed quartz, whose surfaces (*c* and *c′*) are cut perpendicularly to the optic axis of the crystal. Taken together they form a plate bounded by parallel surfaces, and they can be made to slide on one another by means of a rack and pinion, *v*, so as to increase or diminish its thickness at will. One of the frames in which these is fixed has a scale, *l*, and the other a vernier, *n*. When the zero of this corresponds to the zero on the scale, the left-handed rotation of the two prisms is compensated by a plate of right-handed quartz, *p*, and the field then appears of an uniform colour, but as soon as the prisms are moved this compensation ceases, and the two halves become differently coloured. The same effect is produced by putting a solution of sugar into *o o*. The screw *v* is then turned till the effect of the sugar is counterbalanced and the amount of rotation read off on the scale. At this end *a* is a telescopic adjustment, to enable the division between the two halves of the quartz to be clearly seen.

In using this instrument, the end *b* should be placed opposite the brightest part of a lamp flame, and it is advisable to cover the flame with an earthenware cylinder having an aperture which just admits the end of the saccharimeter, so as to shut off all light except that which passes through the instrument. The zero of the vernier having been placed opposite that of the scale, the operator looks into the end *a*, and adjusts the telescope till the dark line in the centre of the field is clearly defined. If the two sides of the field are of exactly the same tint, he may proceed with the operation, but if they are not, he must adjust them by means of a screw and key, which are not represented in the engraving. The tube is then to be filled with the fluid, to be examined and its end closed by a piece of glass and a metal cap, which should not be screwed too tightly. The fluid must be transparent, and as colourless as possible. A light yellow colour does not interfere with the accuracy of the determination, but a red or brown colour impairs it seriously. Three tubes, 1, 2, and ½ a decimeter in length, are generally supplied with each instrument, and the longer the tube used, the more exact is the determination. Dark fluids may be examined in the shorter tubes, but if very dark they should be diluted before examination. The tube is then placed in the space *o o*, and the rack *v* is turned till the two halves of the field present exactly the same tint. By turning the prism *a*, different colours of the field may be obtained; a pale rose colour is that in which differences of the two halves can be most readily observed. The distance to which the zero of the vernier has been moved from that of the scale to one or other side, indicates the amount of dextro- or lævo-rotation. The compensator is so graduated that each degree of the scale corresponds to one gramme of sugar or albumin in 130 cub. cent. of fluid when a tube one decimeter long is used. When tubes of a different length are employed, the number of degrees must be divided by the length of the tube in order to find out the strength of the solution. As sugar and albumin rotate the rays in a different direction, their amount cannot be determined when both are present in a solution, the instrument then indicating merely the difference between their rotating power. In such a case the albumin must be removed and the amount of sugar determined. The difference between the rotation caused by the sugar alone and the sugar and albumin together, will then of course give the rotation due to albumin. This instrument may also be used for distinguishing between substances, such as albuminous bodies, which nearly resemble each other in their general characters and reactions, but have different powers of rotation or specific rotation. *The specific rotation* of a substance is the extent to which a solution of one gramme in one cubic centimeter, contained in a tube one decimeter long, will rotate a ray of light passing through it. To indicate rotation of light to the right, a + is prefixed to the number of degrees through which the beam is turned, and a — to indicate rotation to the left. The specific rotation of sugar is + 56°, that of albumin — 56°. To find out the specific rotation of any substance with the saccharimeter, the following formula is used (Hoppe-Seyler):—

$$(a\ j = \pm 56^\circ \frac{a}{pl}$$

$(a)j$ is the usual symbol for the specific rotation for yellow light, a is the rotation indicated on the scale, p the weight of the substance in grammes contained in 100 cub. cent. of the solution, and l the length of the tube employed. The specific rotation of different albuminous bodies for yellow light, as given by Hoppe-Seyler for serum albumin, is — 56°, and for egg albumin — 35°·5. The conversion of serum albumin into acid albumin by phosphoric or acetic acid increases its specific rotation to — 71°, and a solution in hydrochloric acid has a rotation of — 78°·7. Serum albumin treated with caustic potash has a rotation of — 86°; egg albumin, — 47°; and coagulated egg albumin, treated in the same way, — 58°·8, for yellow light.

LIST OF THE MOST IMPORTANT INSTRUMENTS AND APPARATUS REFERRED TO IN THIS WORK, WITH INFORMATION AS TO WHERE THEY CAN BE OBTAINED.

I. HISTOLOGY.

MICROSCOPES.—*Hartnack & Co*, 21, *Place Dauphine, Paris; and Carl Zeiss, Jena*, for the best; *Bénèche*, 17, *Grossbeeren Strasse, Berlin; and Wasserlein Bessel Strasse, Berlin*, for good instruments at a lower price.

WARM STAGES, INJECTING APPARATUS, SYRINGES AND CANNULÆ, FINE SCISSORS AND FORCEPS, STEEL CLIPS, &C.—*Hawksley*, 4, *Blenheim Street, Bond Street.*

HATCHING OVEN.—*Jordan, Wells Street, Oxford Street.*

SOLUBLE PRUSSIAN-BLUE.—*Marquart & Co., Bonn.*

GELATIN.—*Crosse and Blackwell, Soho Square.*

CHEMICALS.—*Hopkin and Williams*, 16, *Cross Street, Hatton Garden.*

II. PHYSIOLOGY OF THE CIRCULATION AND RESPIRATION.

GAS PUMPS.—*Alvergniat, Fabriquant d'Instruments de Physique, Paris (close to the Collège de France); Dr. Geissler, Mechaniker, Bonn; Luhme & Co.*, 51, *Kur Strasse, Berlin.*

SPRENGEL'S PUMP, FRANKLAND AND MACLEOD'S APPARATUS, RECIPIENTS AND OTHER APPARATUS FOR ANALYSIS OF BLOOD GASES.—*Cetti & Co.*, 11 *Brooke Street, Holborn.*

KYMOGRAPH, ARTERIAL SCHEMA, CARDIOGRAPH, MAREY'S TYMPANA AND LEVERS, RECORDING STETHOMETER; CZERMAK'S RABBIT SUPPORT; MANOMETER, AND SUPPORT FOR COATS' APPARATUS; DELICATE THERMOMETERS; ANEURISM NEEDLES, BLUNT HOOKS, CLIPS, SCREW CLAMPS AND OTHER INSTRUMENTS.—*Hawksley*, 4, *Blenheim Street, Bond Street.*

BERNARD'S HOLDER.—*Guéride, Fabriquant d'Instruments de Chirurgie, Paris.*

FICK'S SPRING KYMOGRAPH.—*Weber, Mechaniker, Rothscheiben Strasse, Würzburg.*

SPHYGMOGRAPH.—*Breguet*, 39, *Quai de l'Horloge, Paris.*

BUNSEN'S COMPRESSED AIR WATER-PUMP. — *Desaga, Universitäts-Mechaniker, Heidelberg.*

Rosenthal's Apparatus, Pettenkofer's Absorption Tubes, Bunsen's Aspirators, &c.—*Cetti & Co.*, 11, *Brooke Street, Holborn.*

Calorimeter.—*Jordan, Wells Street.*

III. PHYSIOLOGY OF THE NERVOUS SYSTEM.

Galvanometers and Shunts, Du Bois Reymond's Induction Apparatus, Rheochord, Commutator, Wippe and other Electrical Apparatus.—*Elliott & Co., St. Martin's Lane.*

Moist Chamber, Marking Key, Marey's Myograph, &c.—*Hawksley*, 4, *Blenheim Street.*

Batteries.—*Lund*, 12, *Brooke Street, Holborn.*

IV. DIGESTION AND SECRETION.

Gastric and other Cannulæ.—*Hawksley*, 4, *Blenheim Street.*

Bunsen's Regulator.—*Cetti*, 11, *Brooke Street.*

Water-baths for Digestion.—*Jordan, Wells Street.*

Beakers, Test-tubes, and Glass Apparatus.—*Powell and Sons, Whitefriars Glass Works.*

Chemical Apparatus.—*Griffin*, 22, *Garrick Street, Covent Garden; and Jackson, Bishopsgate Street Within.*

Soleil's Polarization Apparatus, as Adapted by Ventzke, for the Quantitative Determination of Grape Sugar and Albumin.—*Luhme & Co., Berlin.*

Apparatus for Volumetrical Analysis, and Titrated Solutions.—*Sutton & Co., Norwich.*

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